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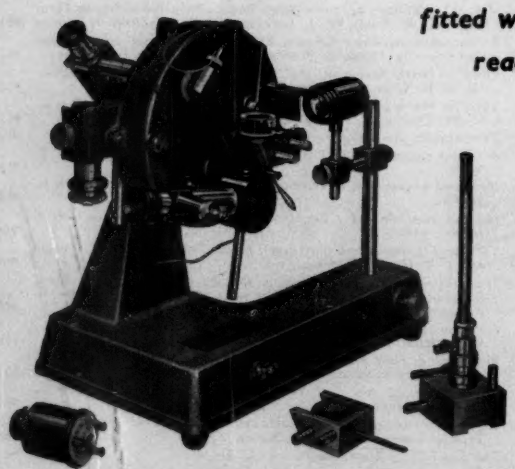

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REDUNDANCY IN INDUSTRY

IN a notable speech opening a long debate on the economic situation and wages at the Trades Union Congress on September 4, Mr. Alan Birch, chairman of the Congress's Economic Committee, after remarking that the trades unions' record of productivity was good, asked whether it was not true that too often co-operation and new methods in industry were regarded by the unions not so much as a positive thing for their own benefit, but as a concession to the employers. He suggested that the trades unions should use their organization to demand efficient organization and management, as well as to obtain a fair share in remuneration. He recognized that this meant a positive rather than a negative approach and that if the trades unions were to deal with the new problems posed to-day there must be a break with the approach developed when the unions were being built up and were on the defensive. Their industrial, economic and emotional approach must be based on the experience of the past two decades and not on that of previous ones.

Mr. Birch's views appeared to find little support in the general body of the Congress, but they are firmly supported by a study of redundancy, one of the problems specifically mentioned by Mr. Birch, which the Acton Society Trust has recently published*. This introductory survey of problems and practices associated with redundancy has been sponsored by the Society as part of its general plan of work, which seeks to obtain accurate factual knowledge about important contemporary developments in our industrial society so as to facilitate our understanding of the effect of such developments on individuals—their lives, their liberties, their values. Some of these problems arise from the increasing dependence of our industrial efficiency upon change and flexibility, and it is here that what have come to be known as problems of redundancy arise. Moreover, these are often very real human problems which must be handled with sympathy and understanding if, in a liberal society, progressive economic efficiency is to develop soundly.

In this study of the causes of redundancy, which is clearly distinguished from unemployment, its relation to the mobility or immobility of labour, its effect on individuals, and the measures which are being or could be taken to reduce the costs, material and psychological, which redundancy imposes, the Acton Trust is clear that the trades unions have a major responsibility. Redundancy is the involuntary loss of a job through no fault of the worker concerned. Since the redundant worker may be able to go straight into another job, it is not necessarily the same as unemployment; moreover, the unemployed will include some who have been dismissed for personal defects or have left of their own accord, and yet others who are seeking their first job. The problems of redundancy may indeed be sharpened by a depression, but throughout this survey it is assumed that full employment policy, in the sense of the maintenance of a general balance between the supply and demand for labour, is being successfully pursued.

Traditionally, the primary responsibility for coping with the effects of redundancy is the worker's own, and, in spite of arguments that it should not be his exclusively, it remains true that the responsibility for managing one's own life is inherent in the preservation of individual freedom. Nevertheless, it is consistent with this, and realistic, to argue that the trades unions have a major responsibility: on one hand, they should accept the inevitability of some redundancy, especially when a forward wage policy has produced an economy of increasingly costly man-power; and on the other, they should accept the responsibility for co-operating in handling redundancy so as to minimize hardship. Nevertheless, the survey fails to suggest that trades unions have been unreasonably backward in negotiating redundancy agreements. This may have been due to the backward view which Mr. Birch condemned, but it could also be due to the belief, held by employers as well as trades unions, that redundancy is often better handled at a local, rather than a national, level.

The Acton Society Trust makes two suggestions as to how the trades unions might help to reduce hardship and to encourage the best utilization of our resources. First, it suggests that the unions might pay greater attention to securing some form of protection against redundancy for their members, so as to safeguard them and make them less fearful of technical change. For example, the unions might negotiate a plan for compensation as a form of deferred pay, possibly safeguarded by establishing a redundancy fund. Secondly, and highly pertinent in the context of recent events, it is suggested that some attention should be given to the position of coloured people when redundancy occurs. The Trust regards this as a question about which the unions should make up their minds and then educate their members.

By and large, so far as the unions are concerned, their immediate contribution to the problem of redundancy seems to be a matter of education and a new outlook, and it once again indicates the critical importance of education, and especially of technical education. For the present, the survey stresses rather the responsibility of government and of industry. The former is regarded as having three aspects: the framing of general institutions and enactments where these impinge on industry so as to encourage rather than hamper the mobility of labour; the active assistance of mobility where public agencies can do so effectively; and the guaranteeing, as part of the general obligation of the Welfare State, of a minimum standard of living,

* Redundancy: a Survey of Problems and Practices. Pp. vi+56. (London: The Acton Society Trust, 1958.) 5s.

through unemployment insurance and national assistance.

It is on the degree of responsibility of employers that opinions differ most widely, but before considering the Trust's comments here and its further suggestions regarding the role of the Government, it is as well to consider what is implied in the objectives of a redundancy policy. The evidence of this survey suggests that hardship can best be reduced by paying special regard to the most vulnerable groups, particularly those in their late forties and early fifties, assisting people to get new jobs and helping them in the transition period. The second objective, that of encouraging the best utilization of man-power, can be achieved partly by making it easier for people to be mobile, especially geographically, and partly by bringing work to the workers where this is feasible.

The specific ways in which it is suggested that Government could further the achievement of these objectives are, first, through legislation, which requires employers to take such action as paying compensation or giving longer notice, the latter being possibly the more desirable. It might also be worth while to consider the feasibility of an insurance scheme to meet the contingency, and such a scheme might encourage a more responsible policy to redundancy and lay the real costs of uncertainty and instability where they properly belong. Secondly, changes in unemployment-pay, such as a higher initial benefit or the introduction of different contributions and benefits based on difference in earnings, as is done in Germany and Canada, is suggested, while fuller use and less-rigid application might well be made of the existing schemes for payment by the Ministry of Labour, in 'designated' circumstances, of removal expenses, fares and lodging allowances.

In its remaining suggestions for the role of Government, the pamphlet touches on joint responsibility with the employers and with the trades unions, and in both cases we are concerned with education. The publication, "Positive Employment Policies", issued earlier in the year by the Minister of Labour, is an example of what Government can do to exhort or educate employers to adopt responsible and adequate policies, while even more specifically the Ministry's training scheme depends on the goodwill of both the trades unions and the employers. The use of the scheme depends indeed on a more co-operative and progressive attitude on their part; it is a matter not simply of training or re-training the workers affected but of educating both unions and employers to face the responsibilities and new demands of technological change and development.

As regards the role of the employer, the Acton Society Trust takes the view, widespread and gaining ground among employers, that a firm is a social as well as an economic organization, with responsibility not only to its shareholders, but also to its workers, its customers, and to the wider community of which it forms part and through which it exists. In the light of this conception, three main points are made. First, with careful planning of man-power, the effects of redundancy can often be avoided or reduced, and even where this is not possible, longer notice can be given. Secondly, the employer, and especially the larger employer, can obtain information far more readily than the individual worker and therefore do much to assist and supplement the Ministry of Labour's efforts to find him other employment. Thirdly, in employing a man, employers incur not simply a liability to pay him for his work as it is performed week by week, but equally a liability to build up a reserve fund in respect of each man to cover either the employer's contribution to a pension or some form of compensation in the event of termination of employment before pension age is attained. The greatest need seems to be to endeavour to protect the older and most vulnerable employees through early pensions, or higher compensation where it is necessary to discharge them.

Employers can obviously help to reduce hardship by making, as some companies do, tide-over payments until a man has found a satisfactory job, and the survey finds some evidence of the great lengths to which some employers will go to ensure that the basis of dismissal is fair. It seems equally clear that there is room for such personal interest or touch and that its existence can make a most valuable contribution to the removal of hardship and any sense of dissatisfaction.

Finally, the pamphlet discusses the implication of the Government's policy for the distribution of industry and its bearing on mobility. Here it makes some sound remarks on the community aspect of the problem and points out that the failure to deal rationally with the reform of local government has hindered the dealing with the distribution of industry on its economic merits. It is too early to judge the effect of the Development of Industry (Industrial Finances) Act, though it is regarded as a useful addition to a development area policy which has become too rigid to meet present-day requirements.

Here, as well as the outstanding impression left by this preliminary survey, is the continuing need for fresh thinking and readjustment to changing conditions. Largely it is true that the initiative must rest with the Government, but the task of education required both to secure the essential mobility, the re-training and adjustment of labour to new occupations, the removal of restrictive and obsolete practices that hinder transfer and adjustment, is immensely varied and demands clear understanding and close co-operation on the part of both the unions and the employers. The vital importance of both technological and technical education is once again to be seen, both as a contribution to the supply of management of the high quality and wide outlook demanded, and to the supply of technicians and craftsmen, as well as technologists, of the flexibility and adaptability which an age of rapid technical change requires. It is, above all, on the vision and energy and urgency with which the expansion of technical and technological education is pursued that the ability of Britain to solve the problems of redundancy and to maintain its industrial efficiency ultimately depends.

SCIENCE TEACHING IN SECONDARY SCHOOLS

The Teaching of Science in Secondary Schools
Compiled by a Joint Committee of the Incorporated Association of Assistant Masters and the Science Masters' Association. Second edition, revised and reset. Pp. ix+274. (London: John Murray (Publishers), Ltd., 1958.) 17*s*. 6*d*. net.

THE original edition of this book dealt, in a practical manner, with every aspect of science teaching in the grammar type of secondary school. In the present edition every chapter has been revised thoroughly and the work is, in fact, a new book.

The original edition was the outcome of several years work by a committee which had the help of two hundred corresponding members. The present edition likewise is the fruit of the work of several years. Its appearance is timely, since so much thought is being devoted to the extension of science-teaching and to the place of science in the school curriculum. In particular, the new edition will prove most valuable because of the immense fund of practical experience available to the compilers on the design and equipment of laboratories and science rooms. When new schools are built or old schools are improved, teachers who act in a consultative capacity will find in this book a useful guide towards a solution of the problems which beset modern teaching laboratory design.

The work begins with a short history of science-teaching, and it is hoped that the future will see a separate volume dealing with the 'comparative' history of science teaching. It is useful to envisage the present system, with its difficulties and imperfections, through its history.

In the following chapter the aims of science-teaching are clearly stated. This is important, since so many so-called aims of education are entirely idealistic and theoretical. A pleasing feature of the book throughout its pages is that it keeps its feet firmly on the ground. Whereas the cultural value of science is stressed, its place in the competitive extra-mural world of technical achievement and economic survival is not forgotten.

Subsequent chapters deal in detail with the time desirable and available each year of the school course for teaching science, and the practical needs in the form of laboratories, demonstration, store and other rooms for schools of various sizes; plumbing, lighting, heating, ventilation, water, gas and electricity supplies are all considered in an expert manner. Even the acoustics of science rooms, floor materials, wall surfaces, doors, hatches and blackboards have reasonably adequate treatment.

There follows a chapter packed with information, presented succinctly, on apparatus and its maintenance, materials and safety precautions. Various methods of science-teaching are discussed and there is a very useful chapter on advanced-course work and preparation for university entrance. This is particularly valuable since the sixth forms of many smaller grammar schools are not adequately equipped, in materials and staff. A useful warning is given concerning the dangers of neglecting the mathematical background of the school sciences. Other sections deal with examinations, visual and other teaching aids, science in rural schools, first aid in the laboratory, the supply and training of science teachers, the science teacher and the law. The work concludes with a comprehensive bibliography.

There are some sensible and encouraging remarks on the training of science teachers, though one wonders where men and women with all the qualities and qualifications, given as necessary for the teacher of potential teachers, can be found.

The aims of 'methods' lecturers in science are two-fold: "first, there is the immediate aim of suggesting to the beginner appropriate methods of approach, describing good techniques for handling material so that it may make its full contribution to the education of the pupils. Secondly, the 'methods' lecturer is often faced with the task of doing his best to correct the unfortunate bias of some university courses. For instance, in many universities students are turned out without any knowledge of laboratory technique and are entirely ignorant of the subject in which they have been trained. The university course often concentrates on teaching skills and techniques applicable mainly to elementary research. The 'methods' lecturer endeavours to correct this by emphasizing the social and historical bases of science and by stimulating thought on more fundamental problems concerning the implications of science".

Not only should every teacher and potential teacher of science know this important book; but also it should be read by every head teacher and educationist with administrative, authoritative or senior status.

W. L. SUMNER

ENZYMES AND RELATED TOPICS

Advances in Enzymology and Related Subjects of Biochemistry
Edited by F. F. Nord. Vol. 18. Pp. v+435. 9 dollars. Vol. 19. Pp. v+457. 9.85 dollars. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1957.)

THESE two volumes contain a pleasing selection of the several types of review for which "Advances in Enzymology" have acquired such an excellent reputation. Of the fourteen articles, two are detailed accounts of work on a single pure enzyme; seven discuss enzymes in connexion with particular metabolic systems, such as the tricarboxylic acid cycle, photosynthesis, or nitrogen metabolism; three are not specifically concerned with enzymes, but with related topics, namely, cytochromes, lipoic acid, and deoxyribonucleoprotein; and one deals with a group of enzyme inhibitors. In addition, the compilers of these volumes have followed an early tradition of including an occasional article on a process in which enzymes must surely be involved, although as yet virtually nothing is known of the enzymic stages; in this instance by including an article on lignification.

In Volume 18 the pure enzyme discussed is succinic dehydrogenase. Drs. Singer, Kearney and Massey give a clear account primarily of their own recent work on the purification and properties of this flavoprotein. Particularly interesting is their discussion of the role in this system of the iron associated with the flavin group; they suggest that the iron may be functional in some reactions catalysed by the enzyme but not in others. Next to this article is an excellent review by Dr. E. F. Hartree of the cytochromes in higher plants, most of which, of course,

function enzymically in oxidation systems. It collects together all the work on the occurrence of cytochromes in various plant tissues, from the early classic work of Keilin and his collaborators to the more recent discovery of specialized plant cytochromes such as *f* and b_7. The function of these cytochromes as terminal oxidases, in photosynthesis, and in the transport of ions, is discussed in some detail.

Sir Rudolph Peters, in an account of the "Mechanism of the Toxicity of the Active Constituent of *Dichopetalum cymosum* and Related Compounds", develops the thesis that fluoroacetate and its analogues inhibit carbohydrate metabolism by a process of 'lethal synthesis', the enzyme inhibitor produced being fluorocitrate. The tricarboxylic acid cycle is also dealt with in a review, in French, by Prof. J. M. Wiame, on the importance of the cycle in biosynthesis, primarily in micro-organisms.

A very valuable article is that of Dr. H. L. Kornberg on "Pyrophosphorylases and Phosphorylases in Biosynthetic Reactions". It collects together into a logical system the considerable number of enzymes which have come to light in recent years which act on phosphorylated compounds at the phosphate linkage, either by transferring the phosphate or the remainder, or in a more indirect way bringing about the synthesis of a new compound at the expense of the energy of the phosphate linkage. There is a limited amount of detail about individual enzymes in the group, but very useful discussion of the possible intimate mechanisms involved.

Other articles in this volume are by Dr. L. J. Reed on "The Chemistry and Function of Lipoic Acid", by Drs. Butler and Davison on deoxyribonucleoprotein, by Dr. W. O. James on the respiration of higher plants, and by Drs. Schubert and Nord on lignification in higher plants.

Turning now to Volume 19, the article on the properties of a pure enzyme is by Drs. Kimmel and Emil Smith, on papain. This enzyme is of considerable commercial importance; it was unknown to the present writer, until he read this account, that 500,000 lb. of crude papain, the product of more than 2 million papaya trees, are imported annually into the United States alone. It is also of considerable academic interest, since papain can be easily purified, especially as a mercury salt, and is a small molecule with a single peptide chain of 180 residues. These properties, together with the ease with which quantitative studies of its enzymic activities can be carried out, make it specially suitable for an attempt to relate protein structure and enzyme activity. Prof. Smith has himself made striking contributions to this end; he has shown, in particular, that 120 ± 10 of the 180 residues can be removed from the N-terminal end of the chain without loss of all the enzyme activity. In this article, a plausible reaction mechanism is put forward on the basis of such studies.

Enzymic reaction mechanisms are also discussed at length in the review by Dr. H. S. Mason on "Mechanisms of Oxygen Metabolism". This long and in parts difficult account of enzymes 'activating oxygen', especially those introducing hydroxyl groups into cyclic compounds, contains a good deal of theory and speculation. It should prove very stimulating to those working in this field, and may provoke further experimentation along lines which will clear up the many present difficulties, especially the role of reduced coenzymes I and II in the action of hydroxylating enzymes.

Nitrogen metabolism is reviewed (in French) by Prof. Braunstein, of Moscow. He refutes the common idea that amino-acid metabolism involves simply the oxidative deamination of all amino-acids to give ammonia, which is immediately converted to urea. There is, in fact, a complex pattern of amino-acid metabolism analogous to the glycolytic and tricarboxylic acid pathways of carbohydrate metabolism. As well as a historical survey of earlier work, recent Russian work on the importance of vitamin B_6, together with some comparative studies on deamination, is presented. The activation of amino-acids is discussed, in German, by Prof. Wieland and Dr. Pfleiderer, who, among other things, give a good account of the chemistry of S-acyl derivatives of amino-acids, aminoacyl phosphates, and aminoacyl-adenosine-5′-phosphates.

The remaining article, actually the first in the volume, is by Drs. Vishniac, Horecker and Ochoa on "Enzymic Aspects of Photosynthesis". This contains, as well as a discussion of photosynthesis itself, a good survey of the known location in plants of the enzymes and coenzymes of carbohydrate metabolism as elucidated in animal tissues. This part of the article supplements that of Dr. James in Volume 18 already noted. The Calvin mechanism for carbon dioxide fixation, which has been reviewed elsewhere, is discussed at some length, but the emphasis is usefully laid on the occurrence of the enzymes involved.

Both Volumes 18 and 19 of "Advances in Enzymology" were published in the same year, and it is apparently the intention of the publishers to increase the previous frequency of one volume per year. This should enable them to keep them down to the present handy size. All in all, these two volumes maintain the high standard which dates from Volume 1 in 1941.

Edwin C. Webb

5-HYDROXYTRYPTAMINE

5-Hydroxytryptamine

Proceedings of a Symposium held in London on 1st and 2nd April, 1957. Edited by Dr. G. P. Lewis. Pp. xvii + 253. (London and New York: Pergamon Press, 1958.) 60*s*. net.

THIS is an account of a two-day international meeting organized at the instigation of the Co-ordinating Committee for Symposia on Drug Action. It was held at the Wellcome Foundation, London, in April 1957, and was the third meeting of this type, the earlier ones being on histamine and on hypertensive drugs. Nearly fifty research workers took part, and they included pharmacologists, physiologists, biochemists, pathologists and experimental psychiatrists.

5-Hydroxytryptamine is a substance of potent biological activity which is widely distributed in living organisms, being found in plants as well as in various species of animals. It occurs in many tissues, including blood platelets, intestinal mucosa, carcinoid tumours and in a number of vegetable and animal stings and venoms. This book contains a striking picture of the 'sting-print' of a nettle, made by pressing the leaf on filter paper and developing the spots of 5-hydroxytryptamine by chemical means. In this connexion, it is interesting to find that its biological activity is strongly antagonized by extracts

of dock (*Rumex*) leaves. The symposium was divided into a number of sessions (with different chairmen as indicated), which dealt with the occurrence of 5-hydroxytryptamine (Sir Charles Harington), its metabolism and its cardiovascular actions (Dr. W. Feldberg), its other actions—concerned with nerve endings and ganglia, carcinoids, anaphylaxis and gastric secretion (Dr. Marthe Vogt), antagonists to its peripheral and central actions (Dr. B. B. Brodie), ending with a general discussion (Prof. J. H. Gaddum). Each group of papers was followed by its own discussion. It has sometimes been questioned whether, in symposia of this type, the reporting, more or less verbatim, of the spontaneous discussions is worth while. It was certainly worth while in the present symposium, for the discussions were lively, good-natured and free from trivialities but not from humour; moreover, they included a good deal of unpublished experimental material.

The volume is very well produced. It has an index—very brief, but better than none—and there are useful bibliographies at the end of the sections. The work on 5-hydroxytryptamine is scattered over a wide range of journals and it is valuable to have the more important findings collected together and discussed in this volume.

D. C. HARRISON

FOSSIL MAMMALS

Traité de Paléontologie

Publié sous la direction de Prof. J. Piveteau. Tome VI: Mammifères: Évolution. l'Origine des Mammifères et les Aspects Fondamentaux de leur Évolution. Deuxième volume. Pp. viii+962 (1040 figures et 1 planche). (Paris: Masson et Cie., 1958.) Broché, 15,500 francs; cartonné toile, 16,500 francs.

THIS encyclopaedic work treats of the fossil placental mammals, except the Carnivora, Cetacea and Artiodactyla, which come in the first volume, and the Primates, which are allotted a separate Tome VII. Despite the title, there is nothing about the origin of mammals in the present volume, where the histories of some twenty-five orders, separate since the early Tertiary, are recounted in considerable detail, including a description of every genus.

Many of the contents have already appeared in an abridged form in Grassé's "Traité de Zoologie": the article on Proboscidea is reprinted verbatim from that work. Some sections, however, are new, notably J. Viret on the Perissodactyla, which is of a high standard; R. Hoffstetter on the Xenarthra, an excellent account; S. Schaub on the Simplicidentate rodents, which develops in full the classification already used in the "Traité de Zoologie", and R. Saban on the Insectivora, which uses a new classification. Mlle. C. Dechaseaux has contributed useful articles on endocranial casts, including some new material. There are also some interesting articles by Prof. Piveteau on drawings made by Palaeolithic man.

Together, these articles form a compendium of existing knowledge which will be of great value as a work of reference, particularly to research workers. The numerous illustrations will be especially useful to the advanced student, and the extensive bibliographies provide an excellent guide to the literature. However, the detailed treatment tends to obscure the evolutionary picture: the reader becomes overwhelmed by generic distinctions, and the authors have, on the whole, not taken sufficient opportunity to present synoptic and critical interpretations of the data. They are not altogether to blame: a considerable proportion of the fossil mammals are so poorly known that there is little on which to base an opinion of their relationships, and in many cases where the direction of evolutionary change has been established it has not been analysed functionally and ecologically.

Perhaps one of the principal functions of a survey such as this is to reveal gaps in knowledge and suggest lines for future research. It is not surprising that the phylogeny of the rodents and insectivores should be much less well understood than that of large forms like the Perissodactyla, but it seems anomalous that the order Proboscidea, where fossil material is relatively abundant and the general direction of evolutionary change is well known, should be classified on a frankly artificial basis. The predominance of teeth and jaws in the interpretation of fossil mammals becomes very evident when glancing through the illustrations in this volume, but functional interpretations of the dentition are noticeably lacking.

A work on so large a scale must inevitably be somewhat out of date by the time it is published. Few papers that have appeared since about 1955 have been incorporated fully, though a number are included in the bibliography. Recent work indicates that many of the primitive early placentals that have hitherto been classed as Insectivora are not closely related to the existing members of that group.

P. M. BUTLER

THEORETICAL GENETICS

An Introduction to Genetic Statistics

By Prof. Oscar Kempthorne. (A Wiley Publication in Applied Statistics.) Pp. xvii+545. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1957.) 102*s.* net.

THE division is already familiar of physics—and physicists—into the theoretical, or mathematical, and the experimental; and it is becoming so in chemistry, too. Among the biological sciences, there can be little doubt that genetics will be the first to go this way and indeed there have been signs that it has begun to do so, for mathematical treatments of theoretical problems are no novelty in the genetical literature. Dr. Kempthorne's book marks a further significant step in this direction.

In so far as the title, "An Introduction to Genetic Statistics", suggests an account of the statistical methods used in tests of significance and the estimation of genetics parameters, it could be misleading. True, these methods are described, at times in some detail; but the bulk of the book is devoted to the mathematical development of genetical concepts, theories and problems. Mating systems, inbreeding, and population problems are all covered, often in a wealth of detail. Thus inbreeding systems are handled both by what may be termed the direct method, which Malécot has largely developed, and by matrices, these being used as an introduction for the geneticist to matrix algebra. To many, however, the great appeal of the book will be its very full treatment of the theory of biometrical genetics both in populations and in the descendants of deliberately crossed inbred lines. The treatment is always as general as the author can make it, often going well beyond anything that has yet been thrown up by experiment, and

indeed a considerable body of new work is here presented for the first time. Some topics, for example genotype-environment interactions, do, however, appear thus far to have eluded Dr. Kempthorne's interest.

The author says that "the prerequisites of the book are essentially nil if . . . all readers will have had high school algebra". He surely over-estimates either the content of "high school algebra" or the progress achieved by the average geneticist during his school days. Few, in fact, will fail to find Dr. Kempthorne's text to be hard going, and many geneticists will undoubtedly give it up in despair. They will, however, be the losers if they do so. It is essentially a theoretical mathematical treatise: arithmetic is seldom used and never where algebra can be made to serve. Experimental data are at a discount. The level of genetics assumed appears to be lower than the level of mathematics, and the genetical definitions are commonly less precise than the mathematical. The mathematically more elementary chapters are so much more scrappy than the advanced that one inevitably feels the author to have found them so much less interesting to write. The occasional biological gloss, as where a footnote asserts that bees, oysters and snails may be self-fertilizing like plants, gives an impression of archness rather than of accuracy or understanding. But this should neither surprise nor irritate, for Dr. Kempthorne is a mathematician. Rather, we should be grateful that he has devoted his mathematics to developing genetics in a way which all but the merest few geneticists can but accept without hope of emulation. The result is neither elementary nor easy. It will seldom be read for pleasure. But it cannot fail to command respect as a source of constant and indispensable reference for all concerned with the theory of population and biometrical genetics; and it cannot fail to make one wonder how long away is the day when we must recognize, albeit regretfully, that theoretical and experimental genetics have become different, though cognate and complementary, disciplines and skills.

Kenneth Mather

ANTARCTIC METEOROLOGY

Meteorology of the Antarctic

Edited by M. P. van Rooy. Pp. xi+240+8 plates. (Pretoria: Weather Bureau, Department of Transport, 1957.) n.p.

During the past ten years the South African Weather Bureau has done much for Antarctic meteorology. The Royal Naval Weather Service had shown that a useful synoptic chart could be drawn, and the Weather Bureau finds that in spite of shortage of data from some areas a reasonably good chart can be drawn for the whole of the southern hemisphere, especially during the whaling season when the factory ships make regular reports from the higher latitudes. It has not been able to undertake the labour and expense of issuing a daily analysis, but since 1952 it has published basic data for climatological studies and research in its own journal, *Notos*. One of its aims has been to persuade meteorologists from the northern hemisphere to take a more active part in the work, and the new compendium is intended to give as much help as possible to those engaged on expeditions to Antarctica during the International Geophysical Year. It includes a useful account of the main geophysical features and climates of the Antarctic continent, islands and surrounding ocean and of what is known of the surface currents. It shows where previous meteorological data can be found and deals with the special problems of observing under Antarctic conditions.

Discussion of the available data occupies most of the book. W. L. Hofmeyr, studying the distribution of atmospheric pressure at sea-level, directs attention to the deep circumpolar trough all around the Southern Ocean centred in about 62° S. in summer and 64° S. in winter, and tending to be farther north in the Atlantic sector than in the Pacific sector. The only counterparts in the northern hemisphere are the oceanic lows over Iceland and the Aleutians; elsewhere the effect of the land-masses tends to equalize the north–south gradient. The charts accompanying the work seem to indicate a weak tendency for similar smoothing in sectors of the Antarctic where the continental coast has its strongest north–south trend. It is shown that the anticyclone over the continent should be considered as a prevailing but not uninterrupted condition, since wandering cyclones can enter it.

Dr. Loewe shows that existing information about precipitation and evaporation is inadequate for a study of the balance between them. Apart from the scarcity of observing stations and absence of regular shipping lanes, the difficulties of measuring rainfall at sea and snowfall on the continent are very formidable. E. Vowinckel's study of the climate of the Antarctic Ocean gives a clear indication that the ocean currents and surface temperatures are the predominant factors; the same is true for the islands the climates of which are usefully outlined in the next chapter. Around the coast of the continent the differences between one region and another seem to depend more on varying exposure to continental and oceanic influences and on seasonal changes in the limits of the pack-ice. The variation is particularly great in the Graham Land region. This area is given special attention because of the greater abundance of observations. A pioneer chapter by W. L. Hofmeyr on the upper air conditions shows that the circulation pattern is simpler than that of the northern hemisphere and the heat exchange between the pole and lower latitudes appears to be less effective. A final chapter deals with the history and problem of weather map analysis. In listing the twelve deepest lows that have been recorded south of 50° S., it overlooks one of 927·7 millibars reported by the R.R.S. *Discovery II* from the Bellingshausen Sea on October 30, 1932, second only to 923 millibars reported from Adélie Land in September 1951.

The folding map which accompanies the book is not always identical in detail with other recent publications and its contours, though tentative, may give too positive an indication of the postulated Ross–Weddell Graben. The map overlooks the existence of Peter I Island, which would be a more useful station than some of those in Graham Land. The eleven chapters and references constitute a very valuable compendium.

Although Antarctic meteorology is being continually furthered in a qualitative way by skilful arrangement of the growing wealth of data, the work has to be done with rather superficial guidance from physical principles. Even in the northern hemisphere, where there is a close network of observations, meteorologists have emphasized the growing belief that we might do better in the long run by trying to find out what kind

of behaviour the laws of Nature prescribe. In the Antarctic, where it is difficult to get more observations, such guidance seems very desirable. The relatively simple arrangement of land and ocean favours systematic physical study. To make much progress the atmosphere and ocean must be studied together, and qualitatively the Antarctic Ocean is known as well as if not better than the atmosphere. Results of universal importance might readily be obtained there, but the work would involve a joint meteorological and oceanographic expedition to the ocean rather than to the land, including studies of the transfer of heat and momentum, and quantitative investigations of the circulation of the deep water as well as that of the upper air. G. E. R. DEACON

ANNUAL REVIEW OF PHYSIOLOGY

Annual Review of Physiology, Vol. 20
Edited by Victor E. Hall, in association with Frederick A. Fuhrman and Arthur C. Giese. Pp. viii+633. (Palo Alto, Calif.: Annual Reviews, Inc., and the American Physiological Society, 1958. On sale by Annual Reviews, Inc.) 7 dollars.

ACCORDING to the preface in this twentieth "Annual Review of Physiology", the total number of articles of biological interest has increased during these twenty years from 77,000 to 145,000 a year, and the number of papers reviewed has increased during the same period from 4,256 to 5,543. The size of the volumes published has remained almost constant, and the number of words per paper has fallen. These figures show that the authors have been more selective than some people supposed, and present an awful picture of the amount of literature which a physiologist might study if he had the time—nearly four hundred papers a day.

The prefatory chapter is written this year by W. J. V. Osterhout, and gives an interesting summary of some of the fundamental work he has done on the physiology of aquatic animals since he first became interested in the subject sixty-six years ago. About half the remaining twenty articles deal with main branches of physiology—circulation, respiration, reproduction, digestion, excretion, temperature, and the central nervous systems, and provide new instalments of serial stories which are contained in each volume. There is a series of articles on different aspects of Russian physiology; last year Russian neurophysiology was discussed, this year cardiovascular aspects are considered, and next year we are promised an article on metabolism and endocrinology. Those who cannot read Russian are thus given an opportunity of finding out what they are missing. Comparative physiology is represented this year by an article on excretion; last year it was osmotic regulation and next year it will be blood pigments and biological clocks.

In addition to these standard subjects, there are various other articles of special interest this year. There are articles on growth by J. A. Russell and A. E. Wilhelmi, on the physiology of the skin by W. B. Shelley and R. P. Arthur, on synaptic transmission by W. D. M. Paton, on the hypothalamus, adenohypophysis and adrenal cortex by G. Sayers, E. S. Redgate and P. C. Royce, and many others by well-known authors.

The editors are to be congratulated on maintaining so high a standard. Some people think that this volume is little more than an uncritical reproduction of a card index; but some of these articles are much more than this and give a balanced picture of the best modern work. Anyone who hopes to keep in touch with a wide field of physiological work will derive benefit from this book. J. H. GADDUM

HORMONES IN BLOOD

Ciba Foundation Colloquia on Endocrinology
Vol. 11: Hormones in Blood. Edited by G. E. W. Wolstenholme and Elaine C. P. Millar. Pp. xii+416. (London: J. and A. Churchill, Ltd., 1957.) 56s. net.

STARLING defined a hormone as a substance produced in one part of the body to be carried by the circulating blood to another part, where it elicited a physiologically important response. He believed hormones to play an important part in the mechanisms responsible for the co-ordination of function in the body, and time has emphasized the soundness of this belief. Since the time when Aschheim and Zondek found that the urine of pregnant women contained gonodotropic and œstrogenic substances, there have been got from all sorts of urine many materials which possess activity similar to that of extracts of endocrine glands; but, unfortunately, it has not always been remembered that the substance in urine is not necessarily that secreted by the endocrine gland. Moreover, chemists have synthesized substances, for example, diethylstilbœstrol, which, weight for weight, are more physiologically active than hormones when injected into animals or man. This fact has in some instances turned attention from natural hormones to cheap and easily obtained substitutes which may be of particular therapeutic value. The sub-title of the colloquium on which this book is based could have been "Back to Blood", since a study of the hormonally active substances in blood is likely to be more relevant to the problem of what is a hormone in terms of Starling's definition than the identity of active substances present in urine or solely the product of the chemist's ingenuity.

But, as often happens, a step nearer to the problem that can be formulated brings to light others that previously were not so clear.

In some instances, perhaps in many, the substance circulating in the blood is not identical with that which brings about a physiological effect in the tissues, that secreted by the endocrine gland being altered before entry into the tissues where it acts. Thus, Starling's definition may need extension and expansion; but definitions, valuable though they be, often make a cloak for ignorance. This volume is the record of a most thorough and illuminating discussion by experts of how interest has shifted from urine to blood, and is already beginning to move from blood into the tissues, where the processes involved in the control of metabolic functions actually take place. What is precisely meant by the word 'hormone' becomes less certain as a result, but the book conveys excellently the excitement of uncertainty in a rapidly developing and expanding field of research.

F. G. YOUNG

An International Symposium on Aldosterone

Edited by Alex F. Muller and Cecilia M. O'Connor. Pp. x+232. (London: J. and A. Churchill, Ltd., 1958.) 40*s*. net.

IN June 1957, the Clinique Thérapeutique of the University of Geneva organized an international symposium on aldosterone. This book is a record of the papers given and the discussions which they produced. Aldosterone is one of the hormones of the adrenal cortex which is concerned in the regulation of the level of electrolytes, and its secretion appears to be influenced directly by changes in the water and electrolyte balance of the body. One of the main problems in the study of the functions of aldosterone is the difficulty of estimating it with reasonable accuracy in body fluids and tissues, and this question was discussed in the first part of the symposium. In other papers, the biological action of aldosterone was compared with that of other related hormones and work was described on the synthesis of aldosterone by tissues *in vitro*. The second day of the meeting was devoted mainly to the clinical and biochemical findings in patients with an abnormality in aldosterone secretion.

Some of the communications illustrate vividly what a lot of information on aldosterone has been obtained by the use of very small amounts of this scarce material. A particularly striking paper describes how aldosterone labelled with tritium was prepared by incubating correspondingly labelled progesterone with capsule strippings from ox adrenal glands, and how the resulting radioactive aldosterone was then used to study the metabolism of the hormone in the body.

Those interested in the steroid hormone field will be glad that the proceedings of this symposium have been made available in book form, especially as much of the experimental work appears here in print for the first time. It would have been helpful if a figure had been included, showing the structural relationships between the various steroid hormones discussed. All the papers and discussions are in English.

D. C. Harrison

Guide to Unpublished Research Materials

Lectures delivered at a Vacation Course of the University of London School of Librarianship and Archives in April 1956. Edited by Ronald Staveley. Pp. vii+141. (London: Library Association, 1957.) 21*s*. (15*s*. to L.A. members.)

THIS volume, which is complementary to an earlier publication in 1952, "Government Information and the Research Worker", is intended to assist the staff of research libraries and others who collect and use the records of research. The lectures are arranged in two parts. In the first, dealing with science and technology, Mr. A. H. Holloway contributes an introductory chapter on the research report, in which, although the problems of such reports, including the secrecy aspect, when emanating from Government sources are fairly presented, the industrial aspects are largely ignored; nor are they adequately discussed in the following review of materials and sources of the United Kingdom by J. C. Malloch and R. J. Brierley. Mr. A. N. Holloway deals with the research report in North America, while Dr. A. L. Mackay's account of materials and sources of Eastern Europe and China relates largely to published as well as to unpublished sources. A paper by E. J. Offord on the United Kingdom's participation in European Technical Information Projects replaces that delivered in the course.

The remaining seven lectures deal with the social services. Miss Barbara Kyle discusses bibliographical control of unpublished material; L. T. Williams, statistical material and sources; T. Carter, sources in market research; B. D. Copland, the advertising agency and the interchange of information; R. O. Godwin, market research in industrial fields; W. L. Guttsman, who draws largely on published sources, with materials and sources in sociology; and C. M. Franks, with materials and sources in psychology.

From the factual point of view the volume should achieve its purpose and it directs attention to the new function of the Technical Information and Documents Units of the Department of Scientific and Industrial Research as the national centre for receipt of unpublished research materials from other members of the Organization for European Economic Co-operation. Nevertheless, the value of the volume would have been enhanced by a more critical and forward-looking approach to the character of unpublished material. Generally speaking, the adequacy of existing methods of publishing and handling information is accepted without questioning their appropriateness to the needs of the research worker, or to modern techniques of handling and distributing information.

R. Brightman

Elements of Partial Differential Equations

By Prof. Ian N. Sneddon. (International Series in Pure and Applied Mathematics.) Pp. ix+327. (London: McGraw-Hill Publishing Company, Ltd., 1957.) 56*s*. 6*d*.

THIS is essentially a user's book; its main aim is to provide an account of methods whereby special solutions of the famous equations of classical mathematical physics may be derived, and to connect these solutions with physical problems. A good honours student with an interest in applied mathematics should find the book to his taste, and easy to read.

An introductory chapter deals with ordinary differential equations in two or more independent variables; the account of Pfaffian forms is excellently illustrated by Carathéodory's formulation of the basic principles of thermodynamics. The simpler classical processes for the solution of partial differential equations, such as those of Charpit and Jacobi, are discussed in the second chapter, and in the third a survey of second-order equations is given. The remaining three chapters attack the central theme of the book: Laplace's equation, the wave equation, the diffusion equation. Considerable emphasis is laid on the use of Green's function; the treatment is elementary but forward-looking, and should encourage the student to make a deeper study of the connexion with integral equations and integral transforms, a connexion which Prof. Sneddon insists on frequently but cannot pursue in great detail in an elementary account. Pains have been taken to bring in such modern methods as can be explained in reasonably simple terms; fundamental theory is carefully explained, and key theorems are either proved in full, or the need for such proof pointed out and references given.

Physical problems are used to introduce the appropriate equations, to illustrate the theory, to provide worked examples and to furnish a respectable but not overwhelming number of exercises for the student.

T. A. A. Broadbent

PEACEFUL USES OF ATOMIC ENERGY

UNITED NATIONS CONFERENCE AT GENEVA

THE second United Nations Conference on the Peaceful Uses of Atomic Energy far outstripped the first Conference in the number of papers presented and the number of participants. The scope of the Conference was widened by the inclusion of six 3-hr. sessions on controlled fusion research, and this provided some of the most stimulating new information at the Conference. The discussion of this subject was greatly helped by the final stage of declassification of this field of research by the United States and the United Kingdom. The U.S.S.R. presented to the Conference four volumes containing one hundred papers describing the results of their fusion research up to the present time, and also gave some information about their recently completed large-scale fusion devices. New sessions were included on the separation of isotopes by diffusion plants and centrifuges and other methods, but the continued classification of diffusion plant technology reduced the scope of discussion in this field.

An outstandingly successful scientific exhibition was held in a specially built prefabricated building in the grounds of the Palais des Nations. The United States showed at least ten working fusion experiments from Livermore, Los Alamos, Oak Ridge and Princeton, and this was immensely valuable in enabling scientists to grasp the principles of the many different approaches to controlled fusion. The principles of construction and operation of reactors were equally well shown. The U.S.S.R. showed models and photographs of their latest fusion devices, their nuclear propelled icebreaker and their power stations in construction. The French showed models ranging from their pilot diffusion plant to their nearly completed propane bubble chamber for working in high-energy physics. The United Kingdom exhibition was modest in size compared with the United States exhibition but high in quality. It included a one-third scale model of ZETA, a working model of a 'fast pinch' fusion experiment from the Atomic Weapons Research Establishment, a wide range of scientific exhibits, and models illustrating new reactor developments.

A large industrial exhibition was organized in the city, and eighty-four British firms contributed a very impressive range of exhibits.

Many new scientific films were shown at the Conference, including films describing applications of radioisotopes in the United Kingdom; the United States and U.S.S.R. programmes of controlled fusion research; and the first full-scale 100-MW.(E) nuclear power station to operate in the U.S.S.R. This power station has been built together with a new town on some undisclosed site in Siberia. It is ultimately to have six similar units.

Sessions on nuclear power described the operating experience acquired during the past three years on the prototype power stations, Calder Hall and Shippingport, and the progress which is being made in the construction of the world's first nine full-scale nuclear power stations. These are of five main types —the U.S.S.R. graphite-moderated water-cooled reactor, the United Kingdom graphite-moderated gas-cooled reactor, the United States pressurized and boiling-water reactors and the United States 100 MW.(E) fast reactor. The boiling-water reactor is growing in favour owing to the success of the United States experiments. Its capital cost seems likely to be 30 per cent below the cost of the pressurized water reactor power stations.

The Canadians and the Czechs are developing the heavy-water-moderated reactor cooled either by steam or gas or heavy water. The Canadians believe that they can use fuel of natural uranium oxide and achieve fuel costs of about one mil.

The economic sessions showed that the date at which nuclear power costs are expected to achieve parity with conventional power costs ranges from 1962 in the United Kingdom—for the 500 MW. station remote from coalfields—to 1972 or thereabouts in the United States, where nuclear power has to compete with cheap hydro power and low fossil-fuel costs in many areas. Nevertheless, it appears that 15,000 MW. of nuclear power capacity will be installed by 1965–70. In the group of 'underdeveloped countries', India and Japan will take the lead and expect to have 500 and 750 MW. installed, respectively, by 1965.

Descriptions were given of the more advanced types of nuclear power stations which have reached the reactor experiment stage. The reactor using an organic liquid as a moderator is showing promise as a simple low-pressure system with potential application to propulsion and small power units. The aqueous homogeneous liquid uranium salt fuelled reactor of Oak Ridge has had troubles due to failure of welds in the inner core vessel and has now some difficulty with the stability of the liquid fuel. The graphite-moderated sodium-cooled reactor experiment is providing experience in liquid-sodium technology as well as experience in operating metallic fuel elements at higher temperatures than have been used hitherto. The United Kingdom reported on the next stage of development of the United Kingdom power reactors. The advanced gas-cooled reactor, which is being built to operate at fuel element temperatures of 600° C. using uranium oxide fuel, was discussed. The next stage, the high-temperature gas-cooled reactor project, aiming to use all-ceramic elements and to produce outlet gas temperatures of 700° C., was also described. This reactor experiment may be built at Winfrith Heath as an Organization for European Economic Co-operation project. Experiments on the stability and physics of the fast reactor and the construction of a full-scale 100 MW.(E) fast reactor in the United States and plans for fast reactors of 50 MW. and 250 MW. output in the U.S.S.R. were described.

The Conference received reports on the first semi-commercial nuclear propelled ships. The United States *Savannah*, coming into commission in 1960, will use a pressurized water reactor developing 22,000 shaft horse-power. It will carry up to a hundred passengers and is estimated to cost 30 million dollars, so it is far from being economic. The United States expect also by 1962 to install a boiling-water reactor in a tanker in the hope of reducing

capital costs of the propulsion unit to a figure nearer parity with conventional units.

The U.S.S.R. reported on their icebreaker *Lenin*, which will come into commission in 1959. This will have three pressurized water reactors, one stand-by, and 44,000 shaft horse-power will be developed. This icebreaker will open up a 6,000 mile seaway north of the U.S.S.R., an application which would be impossible without nuclear power.

The controlled fusion sessions and the exhibition dealt with experimental devices falling into two main classes. The first class use the pinch effect to confine a hot conducting deuterium plasma in a toroidal or straight tube. Heating is carried out by resistive heating, shock-wave or compression heating, and radio-frequency heating. Under some conditions—when high electric fields are used to maintain the current—large bursts of 5-kV. X-rays were observed, believed to be due to runaway electrons. This produces a serious loss of energy in the plasma. Ion temperatures of several million degrees were reported with electron temperatures much lower. The maintenance of stability and containment of the hot plasma as energy input is increased is the crucial question for the future development of this class of fusion devices.

The U.S.S.R. reported that they had recently completed ALPHA—very similar in size and appearance to ZETA—in a Leningrad laboratory.

The second class of devices is the 'Mirror Machine', in which the hot plasma is also contained by magnetic fields. In one of these devices, the D.C.X., the hot plasma is formed by injecting 600-kV. molecular ions, and stripping them into atomic ions and neutral atoms in an intensely powerful carbon arc. The neutral atoms leave the chamber and the atomic ions are trapped by the magnetic field and convey their energy to the plasma. The U.S.S.R. have a giant version of this just complete, which they call OGRA. In OGRA the molecular ions spiral in a long path to and fro between magnetic mirrors, and it is hoped that they will be dissociated by impact with neutral atoms. The original mirror machine, the pyrotron, heats the plasma by compressing it with magnetic fields—working like magnetic pistons. A delightful small mirror machine from Los Alamos, SCYLLA, compresses a plasma by a very short pulse magnetic field, produced by a condenser discharge in a fraction of a microsecond. The machine was working in the exhibition, producing pulses of neutrons, activating silver foils the subsequent decay of which the admiring crowds could follow.

In a variant of the mirror machine from Berkeley, ASTRON, amperes of multi-million volt electrons are to be injected into a chamber and constrained to follow a path which will produce, by a combination of their magnetic fields with the external field, a stable 'magnetic bottle' which it is hoped will enable a high-temperature plasma to be formed by electron collisions.

The mirror machines, like the pinch machines, will depend for their success on maintaining the tenuous plasma, generating perhaps hundreds of megawatts per cubic metre in a relatively quiescent state. The energy generated may be of the same order as in fission reactors, but instability time constants could be many orders of magnitude smaller.

So far, although lots of fusion neutrons have been produced by these devices, no laboratory has claimed that they are true thermonuclear neutrons. Ion temperatures of several million degrees have been reported, with electron temperatures much lower for some of the pinch machines. Mirror machines have produced electron temperatures of ten million degrees, but ion temperatures are so far unknown. The true thermonuclear neutrons are evidently 'just around the corner', but it is probably a long haul from them to reach the break-even point when energy output from the plasma equals the energy input. Thermonuclear power stations seem at least twenty years away, but the subject has excited such intense interest and experimental activity that predictions based on present knowledge may soon be out-dated.

The biological sessions were well attended, and the material presented was much newer and fresher than in the 1955 Conference when ten years of work was reported. One of the most interesting reports came from the Oak Ridge Laboratory, on the radiation-induced mutation-rate in mice. Hitherto the direct proportionality of gene mutation with dose of radiation has been accepted as a law which holds, irrespective of dose-rates, down to the lowest accumulated doses. W. and L. B. Russell produced evidence for departure from linearity in the relation between mutation-rate and dose for point mutations in the spermatogonia of male mice. Furthermore, they showed that the mature egg cell of female mice is substantially less sensitive to the induction of mutations than the spermatogonia of the male. Similar results have recently been reported by Carter from Harwell. These two facts will probably necessitate a complete reconsideration of the quantitative predictions of the genetic hazard.

The rapidly expanding application of radioisotopes was shown in eight sessions. At the 1955 Conference, Glascock pointed out the potential importance of tritium as a label to supplement the use of carbon-14. This was borne out by a description in this second Conference of the incorporation of tritium into thymidine in order to build a specific label into the nucleoproteins of cell nuclei. Tritium with its very low-energy beta-emission allows very precise localization by the autoradiographic technique. Already this is providing direct evidence on cell nuclear processes which formerly were merely inferred from indirect considerations.

Clinical medicine benefits in the same way from the application of radioactive tracers to problems of diagnosis and the measurement of individual body-functions. The physician is no longer limited to identification of what goes in and what is excreted; he can trace intermediary metabolism *in vivo* by direct counting or by sampling tissues or body fluids. A notable contribution to the Conference was the increasing accent on the use of shorter-lived radioactive isotopes in order to reduce the radiological dose to the subject. Ingenious, too, was the application of oxygen-15, produced in a cyclotron, for investigating malfunction of the lung. The isotope pill for diagnostic purposes predicted by Dr. Libby was demonstrated in the exhibition.

The industrial uses of radioisotopes continue to increase rapidly. The applications to industrial chemistry are among the most important new fields. Sources of radiocobalt in strengths of the order of 100,000 c. will soon be available and will be followed by radiocæsium extracted from atomic wastes. Promising applications to the production of graft polymers, vulcanization, sterilization and food preservation were described and illustrated in the exhibition. Radioactive catalysts were reported to increase yields several fold.

The sessions on fundamental research were instructive and entertaining. The Dubno 12-GeV. accelerator and the Saclay 3-BeV. accelerator were described. Cosmic ray workers flying large stacks of photographic emulsions in Comet proving trials have been able to obtain an enormous amount of new data on the collision of protons ten thousand times more energetic than any which can be produced by the largest planned accelerators. The demonstration, by the orbiting satellites, of the existence of intense belts of 40 MeV. protons, a thousand kilometres or so above the Earth in particular latitudes, was also described. The great question of why pions and nucleons exist with their particular masses and particular interactions was said to remain totally unanswered, in spite of the wealth of new knowledge produced by the accelerators. Dr. Feynman gave an entertaining account of the strange particles, which now total thirty-one. The theoreticians have a new occupation of inventing new rules and waiting to see whether the latest strange particle obeys them.

In the field of nuclear data the present situation leaves no room for complacency, since present reactor technology requires much more precise information which we shall have to work hard to obtain. To help in this, important new tools such as the Harwell linear accelerator and neutron booster providing enormously powerful pulses of neutrons are becoming available.

In the chemical sessions an account was given of the isolation of weighable amounts of berkelium; chemists look forward to going well beyond element 102, aided by expensive reactors with neutron fluxes up to 10^{16} per cm.^{2} per second. The chemical effects of fission fragments appear to be much higher than anticipated, and this may have important technological consequences. There has been a rapid advance in solvent-extraction technology, and long-chain amines and long-chain derivatives of phosphoric acid have been synthesized with highly specific activities.

A great deal of new and valuable information was obtained at this Conference, not only from the lectures but also from the exhibition and films, which made it possible to see in a few days, in an exciting visual way, work proceeding throughout the world. The Conference provided the opportunity for innumerable discussions in small groups to supplement the work of the formal sessions. Future accelerators, the future of modern physics, genetics, plasma physics throughout the world, the future of co-operation in atomic energy in the Commonwealth, in Europe and in the world, and numerous other topics were discussed. The very rich feast taxed our physical and mental endurance.

There has been much informal debate as to whether there should, in three or four years time, be another conference on this scale or larger. On one hand, it was argued that only by having a conference of such wide scope can one persuade Governments to provide the finances for the immensely rewarding exhibitions and to release new information, and also persuade individuals to write up their research in a form suitable for presentation to the world. It was generally agreed that much cross-fertilization between the different ramifications of atomic energy resulted. All this is true enough and we will need to balance this against the great effort and interruption of research work required to prepare for these conferences. After a few months of thinking over the result, we may be better able to decide.

We are, however, all sure that the Conference was immensely worth while. J. D. COCKCROFT

AIR SHOWERS OF SIZE GREATER THAN 10^5 PARTICLES

(I) CORE LOCATION AND SHOWER SIZE DETERMINATION

By M. H. BRENNAN*, D. D. MILLAR and C. S. WALLACE

The F.B.S. Falkiner Nuclear Research and Adolph Basser Computing Laboratories, School of Physics†, University of Sydney

CURRENT observations on the extensive air showers of cosmic rays[1,2] are concerned with two main problems. In the first of these, the cosmological problem, observations are made in order to determine what anisotropies may exist in the directions of incidence of the high-energy primary particles which initiate the showers, and whether there exists a high-energy cut-off in the energy spectrum of these primaries. Such observations have led to the development of large-area detectors of air showers in order that giant air showers, corresponding to primary energies greater than 10^{16} eV., may be detected with appreciable frequency. The second problem is the investigation of the high-energy nucleon–nucleus interactions of the primary and secondary nuclear-active particles in the air, interactions which give rise to the development of the air shower detectable in the lower atmosphere, and here may be considered showers of a more modest size, detectable with sufficiently high frequency with arrays of smaller dimensions.

In order to provide information on the second of these topics, observations were commenced in Sydney at the beginning of 1957 on the properties of showers of size 10^5 particles and greater. Because of the dimensions of the apparatus and of the range of particle densities which can be measured by the detectors, and also because of the rapid decrease of the frequency of showers with increasing size, these

* Present address: Palmer Physical Laboratory, Princeton University, Princeton, New Jersey.

† Also supported by the Nuclear Research Foundation within the University of Sydney.

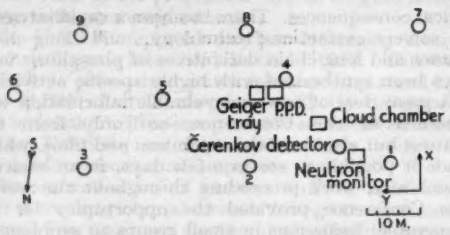

Fig. 1. Plan view of air-shower array. The circles numbered 1–9 represent the liquid scintillation counters. The triggering Geiger trays (not shown) are grouped around the cloud chamber

observations cover an effective range of shower sizes from 10^5 particles to 10^7 particles. Several different instruments are used for detecting the various types of particles in the air showers, for measuring their local density and in some instances for providing information on their directions. These instruments include a large Wilson cloud chamber, an unshielded tray of Geiger counters, a detector of penetrating particles consisting of an array of hodoscoped Geiger counters in a lead pile, a multi-element atmospheric Čerenkov detector, and a device for registering the counts given by a neutron monitor in the period immediately following a shower.

To be able to interpret the results obtained from these instruments, in terms of the structure and development of the shower, it is necessary to know the size of the shower, and the position of its core and the direction of arrival relative to the detecting instruments.

This article describes the array of detectors, the recording gear, the calculations used to obtain the first two of these quantities, and gives some details of the performance and accuracy of the system. The determination of the shower directions will be dealt with more fully in a later publication.

The Array of Detectors

For reasons of cost and convenience, the apparatus has been placed on the roof of the University of Sydney School of Physics, and on three telegraph poles adjacent to the building. The array comprises nine liquid scintillation counters, each of 0·23 m.^{2} sensitive area and 10 cm. depth, arranged in a rather irregular manner, dictated by the topography of the site, inside a rectangle 70 m. × 30 m. The counters are about 20 m. apart (Fig. 1).

These counters are used for giving a measure of the density of the air shower particle flux, by giving an electrical pulse the height of which is proportional to the number of electrons traversing the counter. Some details of their response to the air shower flux have been described elsewhere[3]. As well as the nine density measurements provided by these counters, the time of arrival of the particles is measured in four of the counters, numbers 2, 3, 8 and 9. The difference in these times is used for determining the plane of the shower front, and hence, the direction of the shower axis[4].

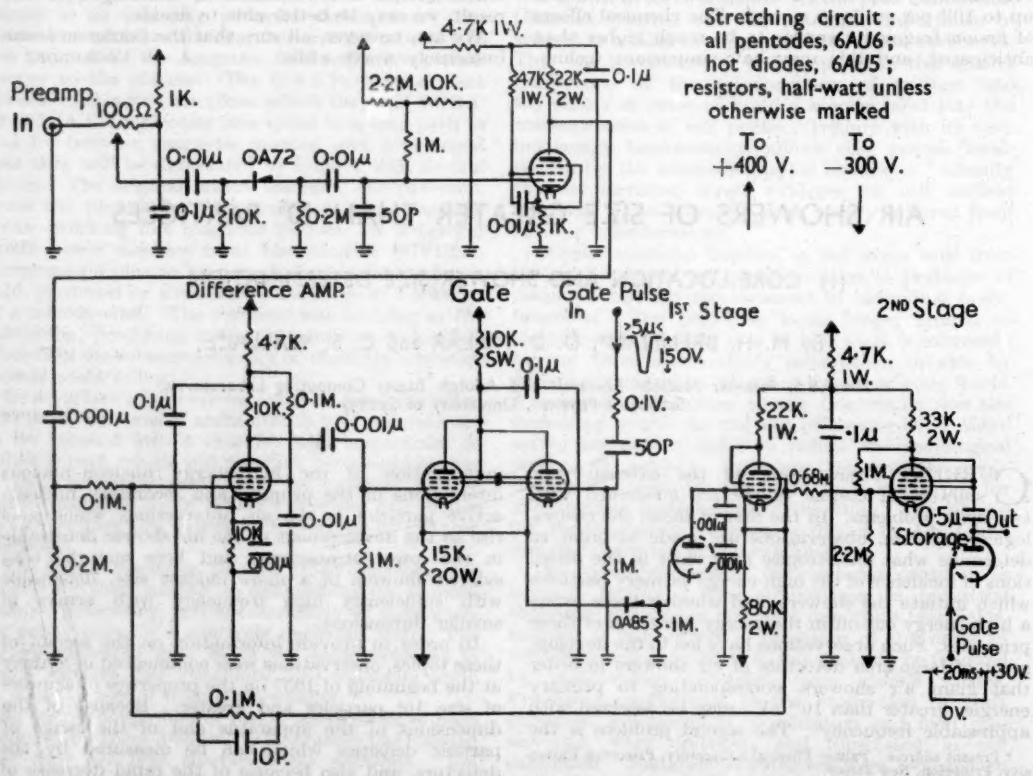

Fig. 2. Stretching circuit used for recording pulse-heights from scintillation counters

Recording the Results

The fast pulses carrying the time-of-arrival information are presented sequentially on the trace of a high-speed oscilloscope, which is photographed. A full description of the details and performance of this gear will be given in another paper.

The pulses carrying the density-of-particles information from the nine scintillation counters are pre-amplified and transmitted via coaxial cable to a central recording station. Here they are further amplified, and gated in coincidence with a master pulse generated whenever a shower triggers the recording gear. The details of the triggering system are described later. The resolution time of the gate is about 5 μsec. The gated pulses are then used to charge up condensers to voltages equal to the heights of the pulses. These condensers have a polystyrene dielectric, and will retain their charge for a period of hours without appreciable leakage. A diagram of the circuit used is given in Fig. 2.

The pulses produced by some of the associated equipment, for example, the multi-element Čerenkov detector and the unshielded Geiger tray, are stored in the same manner.

These stored voltage-levels are then converted one at a time into a binary number in the range 0–40. This covers a voltage range 0–60 V. by 1-V. steps in the range 0–20 V., and by 2-V. steps in the range 20–60 V. The gains of the circuits are arranged so that 1 V. corresponds to the mean pulse size caused by a single relativistic vertical μ-meson traversing the scintillation counter. This level of 1 V. is taken to represent one 'scintillator particle'[2]. The binary numbers thus formed are automatically punched out one at a time on standard 5-hole teleprinter tape. Also included on the tape are binary numbers representing the time of arrival of the shower as day of the week, hour and minute, and a serial number for the shower.

Some of the associated instruments, such as the penetrating-particle detector and the neutron-monitor register, give information directly in 'yes–no' form. This information is stored on thyratrons and punched sequentially on the tape.

The recording of the results from a single shower requires about 2 ft. of tape, and the automatic punching of this tape takes about five minutes. The output tape is allowed to accumulate for a day, during which some sixty or so events will have been recorded. The tape is then fed into the SILLIAC Digital Computer in the Adolph Basser Computing Laboratory for decoding and analysis. A simplified block diagram of the recording gear is given in Fig. 3.

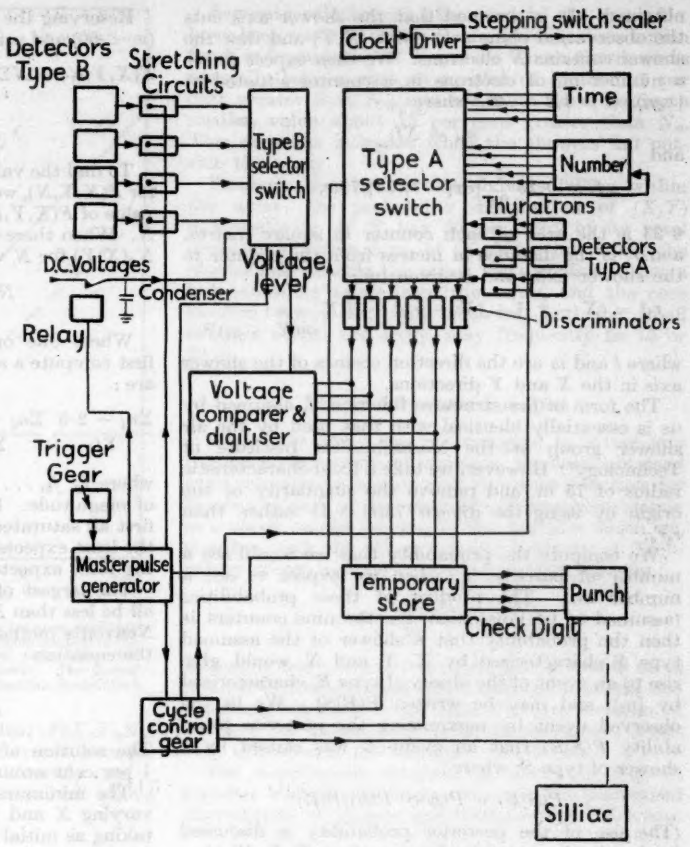

Fig. 3. Block diagram of recording gear

Analysis of the Data

The data tape is input to SILLIAC with a programme which converts recorded pulse-heights into numbers of particles traversing the detector according to the gains of the scintillation counters, which are measured daily by measuring the counting rate of pulses above the supposed 'one scintillator particle' level. An output tape is then obtained which, when printed on a teleprinter, gives a convenient visual presentation of the recorded events. No allowance is made in the programme for variations in the gain of the stretching circuits, which are set fortnightly, and remain constant within about 5 per cent. Also included on the output tape is the direction of arrival of the shower, as determined from the time delays. These delays are input via a manually punched tape.

This output tape contains the results from the various special detectors, besides the particle density information required to determine the core position and size of the shower. This additional information is carried without alteration through the next, and main, stage of the analysis, in which the core position and size are determined.

The analysis is carried out by fitting an assumed lateral structure function for the shower to the observed numbers of particles in the scintillation counters, and varying the assumed centre and total size of this structure function until the 'best' fit is

obtained. It is assumed that the shower axis cuts the observation plane at a point (X,Y) and that the shower contains N electrons. We then expect to see a number m_i of electrons in a counter situated at (x_iy_i), $(i = 1,2 \ldots 9)$, where

$$m_i = Nf_i$$

and

$$f_i = 0{\cdot}23 \frac{1}{2\pi} [\exp -r_i/75]/75(r_i + 1)$$

$0{\cdot}23$ is the area of each counter in square metres, and r_i is the distance in metres from the counter to the shower axis, and is given by:

$$r_i^2 = (X - x_i)^2 + (Y - y_i)^2 - [l(X - x_i) + m(Y - y_i)]^2$$

where l and m are the direction cosines of the shower axis in the X and Y directions.

The form of the structure function, f, assumed by us is essentially identical with that used by the air shower group at the Massachusetts Institute of Technology[6]. However, we take a fixed characteristic radius of 75 m. and remove the singularity at the origin by using the divisor $75(r_i + 1)$ rather than r_ir_e.

We compute the probability that we would see a number of particles n_i when we expect to see a number m_i. The product of these probabilities (assumed to be Poissonian) for the nine counters is then the probability that a shower of the assumed type S characterized by X, Y and N would give rise to an event of the observed type E, characterized by $\{n_i\}$, and may be written $P(E/S)$. We fit the observed event by maximizing the posterior probability $P(S/E)$ that an event E was caused by a shower of type S, where:

$$P(S/E) = P(E/S)\ P(S)/P(E)$$

(The use of the posterior probability is discussed further in the appendix by one of us (C. S. W.).)

$P(S)$ is the *a priori* probability of occurrence of a shower of type S, taken to be proportional to $N^{-2{\cdot}5}$.

$P(E)$ is the *a priori* probability of recording an event of type E. Since $P(E)$ is independent of X, Y and N, it is neglected in the calculation.

We actually compute and minimize the function:

$$F(X,Y,N) = -\log_e (P(E/S)N^{-2{\cdot}5})$$

We take:

$$F(X,Y,N) = 2{\cdot}5 \log_e N + \sum_i [-\log_e (e^{-m_i} m_i^{n_i}/n_i!)]$$

Writing $m_i = N f_i$,

$$F(X,Y,N) = N \sum_i f_i - \sum_i n_i \log f_i - (\sum_i n_i - 2{\cdot}5) \log N + \sum_i \log n_i!$$

Since the recording system can deal only with numbers of particles up to 60, if $n_i = 60$, we use, instead of the probability of getting exactly 60 particles, the probability of getting 60 or more particles, that is

$$\sum_{n=60}^{\infty} (\exp - m_i)\ m_i^n/n!$$

We have tabulated $R(m_i)$ defined as:

$$-\log_e \sum_{n=60} (\exp - m_i)\ m_i^n/n!$$

for $m_i = 0,1, \ldots 80$.

Reserving the subscript i for unsaturated counters $(n_i < 60)$ and using j for saturated counters, we have:

$$F(X,Y,N) = N\Sigma f_i - \Sigma n_i \log f_i - (\Sigma n_i - 2{\cdot}5) \log N + \Sigma R\ (Nf_i) + \Sigma \log n_i!$$

To find the values of X, Y, N leading to a minimum for $F(X,Y,N)$, we first define $F_0(X,Y)$ as the minimum value of $F(X,Y,N)$ obtained for fixed X,Y by varying N. When there are no saturated counters, the value $N_0(X,Y)$ for N which gives this minimum is simply:

$$N_0 = [(\Sigma n_i) - 2{\cdot}5]/\Sigma f_i$$

Where one or more counters are saturated, we first compute a series of approximations to N_0, which are:

$$\frac{\Sigma n_i - 2{\cdot}5}{\Sigma f_i}, \frac{\Sigma n_i - 2{\cdot}5 + 60}{\Sigma f_i + f_a}, \frac{\Sigma n_i - 2{\cdot}5 + 120}{\Sigma f_i + f_a + f_b}, \ldots,$$

where $f_a, f_b, \ldots$ are $\{f_j\}$ arranged in increasing order of magnitude. In doing so we are in effect ignoring first all saturated counters, then all but the one with the least expected number, then all but the two with the least expected numbers, etc.

The largest of these approximations, which must all be less than N_0, is used as the starting value for a Newton's method approximation to the solution of the equation:

$$\frac{\partial}{\partial N} F\ (X, Y, N) = 0$$

The solution of this equation is obtained to about 1 per cent accuracy.

The minimum value of $F_0(X,Y)$ is then found by varying X and Y by a method of steepest descent, taking as initial values for X and Y the co-ordinates of the counter having the largest value of n_i. The best values X_0, Y_0, N_0 (X_0, Y_0) are then printed, together with $F(X_0, Y_0, N_0)$ and estimates of the positive and negative errors in X, Y and N.

These errors are based on the sharpness of the minimum of $F_0(X,Y)$, and are such that the range of values for X, Y and N specified by these errors includes all sets of values (X_1, Y_1, N_1) in the neighbourhood of (X_0, Y_0, N_0) which are more than one-eighth as probable as (X_0, Y_0, N_0). The finite width of the minimum about $F_0(X,Y)$ with variation of N is neglected, as it is always much smaller than the errors in N defined above. Other calculations referred to below have shown that values of X, Y and N in the ranges specified usually contribute at least 90 per cent of the total contribution to the integral:

$$\int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dy \int_{0}^{\infty} dN\ P(S/E)$$

To give a convenient measure of how well this calculated shower fits the observed event, we also compute and print a quantity χ^2 defined by:

$$(s - 3)\chi^2 = [\Sigma (n_i - m_i)^2/n_i]_{0<ni<60} + 2[\Sigma m_i]_{ni=0} + [\Sigma\ 60/m_j]_{nj>60}$$

where s is the number of counters in use, and m_i, m_j are calculated from X_0, Y_0, N_0. The quantity χ^2 is

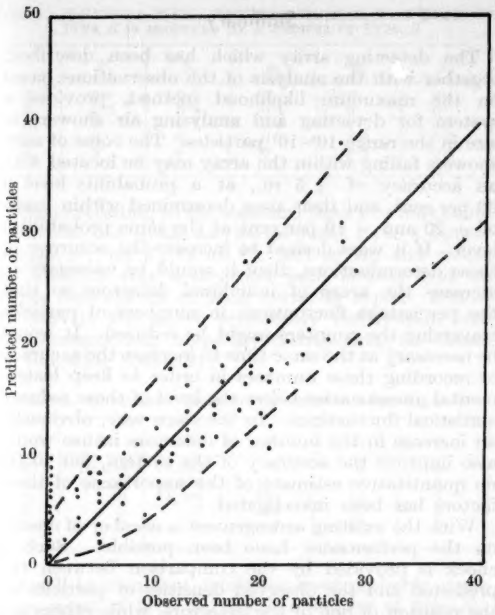

Fig. 4. Scatter diagram showing correlation between predicted and observed numbers of particles in a scintillation counter in the air-shower array. The co-ordinates of each point show the predicted and observed numbers for one shower. The dotted lines represent the 10 per cent limits of a Poissonian distribution

a more convenient measure of fit than $F(X_0Y_0N_0)$ for purposes of comparison with other events, because it has an expectation value of approximately one.

The complete analysis process described above takes about 45 sec. per event.

Accuracy of System

The gains of the scintillation counters are fixed by daily adjustment to an accuracy of about 5 per cent. The pulse storage and digital conversion circuits are calibrated to 5 per cent fortnightly. We therefore expect the particle density measurements to be limited to about 10 per cent accuracy by the instrumentation. This is, however, smaller than the expected Poissonian fluctuations in the measured density. Since we were not sure what effect these fluctuations and instrumental errors would have on the determination of the core position and size of the showers, the following check was carried out: a typical batch of events was analysed as if one of the scintillation counters were unserviceable, that is, the observed number of particles in it was not used in analysing the events. Then, the expected number of particles through this counter was computed from the estimated core position and shower size, and compared with the observed number. The results of this comparison are shown in Fig. 4. It can be seen that the agreement is in general within the expected statistical fluctuations.

The 'errors' in core location and shower size estimated by the analysis programme (see earlier) vary widely from shower to shower. For a shower of size 10^6 particles falling inside the array, the location errors are normally about 5 m., and the errors in size about 20 per cent in the positive direction and 10 per cent in the negative direction.

Separate calculations (discussed in the appendix have been made to obtain the probability distribution for N independently of X and Y. These distributions normally have a peak at a value of N about 5 per cent greater than N_0, and are quite skew, having a median value about 15 per cent greater than N_0. This skewness increases when the showers fall outside the array.

Similarly, for 10^6 particle showers falling within the array, the probability distribution of (X,Y) independently of N has 90 per cent of its total probability within 5 m. of (X_0,Y_0); but when the shower falls outside the array, the distribution has a tail extending away from the array, and the core location errors for such showers, and for small showers within the array, may frequently be 15 or 20 m.

Lateral Structure Function

While this array was not designed to investigate the lateral structure of showers, we have obtained from our results an average structure function for showers of sizes in the range 10^5–10^7 particles, mainly as a check on the validity of the function which we, together with the Massachusetts Institute of Technology group, assume in our calculations, namely:

$$\rho(r) = \frac{N}{2\pi} \frac{\exp(-r/r_0)}{r/r_0}$$

Although our observed distribution is based on a method of core location which assumes a lateral distribution, we have found that the core location of showers, for showers falling inside the array, is not very sensitive to the assumed distribution.

The distribution obtained is shown in Fig. 5, together with the above function and the theoretical distributions of Moliere and Nishimura and Kamata.

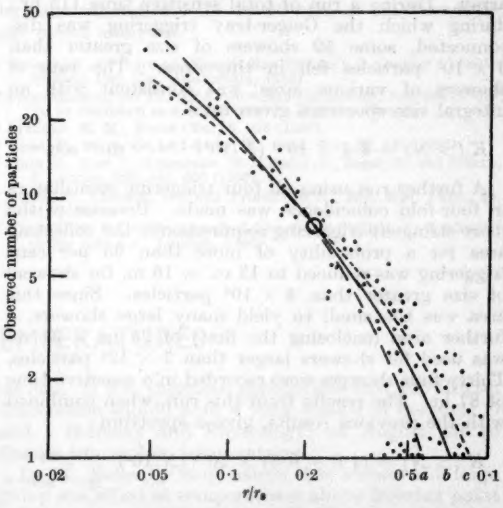

Fig. 5. Scatter diagram showing observed lateral structure function. The co-ordinates of each point represent the distance of a counter from the core of a shower, and the observed number of particles in the counter, normalized to a shower size of 3×10^5 particles. Points are plotted for every counter for 15 showers of sizes in the range 3×10^5 to 10^6 particles. Curve b is the structure function used in our analysis, curve a is the Moliere distribution and curve c is the Nishimura and Kamata distribution for $s = 1{\cdot}4$. Curve b is for a shower size 3×10^5 particles. The other two are normalized to it at the circled point

It can be seen that the agreement with our assumed function is quite good.

As has been found by other workers, the Nishimura and Kamata curve for $s = 1{\cdot}4$ fits the observed distribution very well, but there is a marked disagreement with the Moliere distribution.

Observed Rate of Air Showers

Because this array is primarily intended for the study of individual showers, the arrangements for triggering the recording gear have been designed to give the maximum rate of events likely to give a useful response in the special instruments attached to the array. The array may be triggered either by a four-fold coincidence of one or more particles in each of scintillation counters 2, 3, 8 and 9, or by a three-fold coincidence of more than five Geiger counters firing in each of three trays of 10 counters placed near the cloud chamber (Fig. 1). The area of each counter is 0·02 sq. m. Furthermore, no event is analysed unless at least three of the nine scintillation counters have recorded at least two particles, and no event is analysed if more than six of the nine counters are saturated.

Thus the question of what percentage of showers of various sizes falling in various positions will trigger the array and ultimately be analysed would be very difficult to answer, and we have made no attempt to do so. We therefore cannot obtain information about the rate of occurrence of showers from the majority of our recorded events, and so the rates we have determined have been based on rather poor statistics. However, we have calculated that any shower of size greater than 3×10^5 particles falling inside an area 20 m. × 28 m. centred within the area bounded by three triggering scintillators (at a time when the fourth was not in use) has a probability greater than 95 per cent of triggering the array. During a run of total sensitive time 115 hr., during which the Geiger-tray triggering was disconnected, some 59 showers of size greater than 3×10^5 particles fell in this area. The rate of showers of various sizes was consistent with an integral size spectrum given by:

$$K(>N) = 4{\cdot}5 \times 10^{-9}\,(N/10^6)^{-1.6\pm0.1}\ \text{m.}^{-2}\ \text{sec.}^{-1}$$

A further run using all four triggering scintillators in four-fold coincidence was made. Because of the more stringent triggering requirements, the collecting area for a probability of more than 95 per cent triggering was reduced to 13 m. × 16 m. for showers of size greater than 4×10^5 particles. Since this area was too small to yield many large showers, a further area (enclosing the first) of 25 m. × 31 m. was used for showers larger than 7×10^5 particles. Thirty-four showers were recorded in a sensitive time of 87 hr. The results from this run, when combined with the previous results, give a spectrum:

$$K(>N) = (4{\cdot}0 \pm 0{\cdot}5) \times 10^{-9}\,(N/10^6)^{-1.7\pm0.1}\ \text{m.}^{-2}\ \text{sec.}^{-1}$$

This spectrum is in substantial agreement with that obtained by other workers.

The sizes of the showers have been corrected in this to the sizes which would have been obtained by using Geiger counter detectors rather than scintillation counters. The details of this correction will be discussed in a later article.

Summary

The detecting array which has been described, together with the analysis of the observations based on the maximum likelihood method, provides a system for detecting and analysing air showers of size in the range 10^6–10^7 particles. The cores of such showers falling within the array may be located with an accuracy of ± 5 m., at a probability-level of 90 per cent, and their sizes determined within limits of + 20 and − 10 per cent at the same probability-level. If it were desired to increase the accuracy of these determinations, then it would be necessary to increase the areas of individual detectors so that the percentage fluctuations in numbers of particles traversing the counters might be reduced. It would be necessary at the same time to increase the accuracy of recording these numbers in order to keep instrumental uncertainties below the level of these reduced statistical fluctuations. In the same way, obviously, an increase in the number of detectors in use would also improve the accuracy of the system, but so far no quantitative estimate of the importance of these factors has been investigated.

With the existing arrangement a number of checks on the performance have been possible. Such a check is provided by the comparison between the predicted and the observed densities of particles at the position of one of the detectors, while others are afforded by the agreement between the shower structure function and the shower size spectrum as here reported and the determinations of these by other workers.

We wish to acknowledge the facilities made available for this work by Prof. H. Messel, and the invaluable support given by the Nuclear Research Foundation. Our thanks are also due to the other members of the Sydney Air Shower Group, Messrs. W. Layson, J. A. Lehane, J. Malos, P. C. Poole, M. H. Rathgeber and G. Winterton, for their assistance and co-operation.

One of us (M. H. B.) has been supported in this work by a grant from the General Motors–Holden Co., and another (C. S. W.) by a grant from the Australian Atomic Energy Commission.

Appendix: Use of the Posterior Probability Distribution

If we followed the normal procedure of maximum likelihood analysis[6], we would choose those values of X, Y and N which jointly maximized $P(E/S)$. The result (for events in which no counter was saturated) would be very nearly the same as that obtained by maximizing χ^2, since in this case the difference between $\log P(S/E)$ and $(s-3)\chi^2$ involves only terms of order $m_i^{-1/2}$ and smaller, and terms independent of S. However, analysis along these lines is liable to systematic bias as shown below. Moreover, as some objection to the use of the posterior probability distribution appears in the literature[7], we consider that some justification for its use is required.

Suppose that all showers could be divided into five types denoted S_1, S_2, S_3, S_4 and S_5, and that these different types of showers occurred with relative frequencies $10^4 : 10^3 : 10^2 : 10 : 1$. Suppose, further, that these showers caused events of five types E_1, E_2, E_3, E_4 and E_5 with probabilities as shown in Table 1.

Table 1. VALUES OF $P(E/S)$, THE PROBABILITY THAT AN EVENT OF TYPE E IS PRODUCED BY A SHOWER OF TYPE S

	S_1	S_2	S_3	S_4	S_5
E_1	0·8	0·1	0	0	0
E_2	0·2	0·8	0·1	0	0
E_3	0	0·1	0·8	0·1	0
E_4	0	0	0·1	0·8	0·2
E_5	0	0	0	0·1	0·8

Then according to the standard theory of maximum likelihood, one would ascribe an event E_1 to a shower of type S_1, an E_2 to an S_2, and so on, and would say that in this description each shower had an 80 per cent chance of being correctly typed. If now we convert the above probability distribution into the corresponding frequency distribution, we obtain the following (Table 2):

Table 2. FREQUENCY DISTRIBUTION DERIVED FROM TABLE 1

	S_1	S_2	S_3	S_4	S_5
E_1	80,000	1,000	0	0	0
E_2	20,000	8,000	100	0	0
E_3	0	1,000	800	10	0
E_4	0	0	100	80	2
E_5	0	0	0	10	8

It will be seen that, while the most probable cause of an event of type E_1 is still a shower of type S_1, the most probable cause of an event E_2 is again a shower of type S_1, not S_2. Again, the normal maximum likelihood method would ascribe an event E_3 to a shower S_3 and claim 80 per cent probability of being correct. It will be observed, however, that of all the events of type E_3 recorded, only 44 per cent are produced by S_3 showers; the majority of them are, in fact, produced by S_2 showers.

The chances of correct assignment for E_1 events, which we may reasonably identify with events failing to trigger the apparatus, is 98·8 per cent, but the chances of correct assignment for all other types of events averages only 30 per cent. Thus, if, as is certainly the case, a very numerous class of small showers has a low triggering probability, the frequency of success of the standard method in analysing observed events drops from 80 to 30 per cent.

It will be seen, therefore, that if some classes of showers are vastly more numerous than others, as is the case, and if the probability distribution $P(E/S)$, the probability that an S-type shower will produce an E-type event, is not sharply peaked, then some account must be taken of the *a priori* probability of occurrence of the S-type shower, $P(S)$, in order to avoid a consistent over-estimate of the shower size. This has been done in the analysis of the air shower data by using Bayes's Theorem[6]:

$$P(S/E) = P(E/S)\,P(S)/P(E)$$

The quantity $P(S/E)$ can be meaningfully interpreted only as a relative frequency of the type discussed above. Such an interpretation is not strictly admissible in our case, since we would very rarely indeed see even two events of the same type. However, investigation of the form of the function $P(S/E)$ as a function of S for various different events E has shown that large numbers of events are sufficiently similar to justify the use of this quantity.

It should also be pointed out that we cannot claim freedom from bias in the determination of any quantity which we may wish to estimate about a shower using the analysis programme described. If, for each shower, we had been concerned to find the most probable value of N, the shower size, without at the same time requiring to know the core location, then we should maximize $P(N/E)$ defined as:

$$P(N/E) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} P(S/E)\,\mathrm{d}X\,\mathrm{d}Y$$

This has, in fact, been done by us for a sample of showers as has been mentioned, and the best value of N so determined is, in general, greater by about 5 per cent than is the value computed in the normal analysis. One should, in fact, maximize the probability distribution of whatever single physical result is desired such as shower size, core location, density of particles at a particular detector, and so on. Such a course is, however, impracticable because of the volume of computation involved. The calculations mentioned above for finding the most probable value of N independent of the core location (X,Y), and for finding the probability distribution in the core location consumed some 10–50 min. of machine time per event analysed.

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LIFE AT LOW TEMPERATURES

IN recent decades man has been striving for mastery of chemical, physical and climatic forces, however abstruse or remote from his usual environment. Such mastery, when achieved, would be ineffectual or even dangerous if the biological effects of the same forces had been ignored, either in their impacts on individual plants and animals or in their widest ecological significance. During the past year, temperatures lower than any hitherto recorded in the range below 1° K. have been produced by adiabatic demagnetization and their effects on the properties of matter have been studied. During the same period, Antarctica has been crossed above and the North Pole below its ice cap. It was therefore appropriate that the British Association should hold a joint session of Sections D and I (Zoology and Physiology) on August 28 to discuss life at low temperatures.

Dr. K. Mellanby emphasized that insects of a large group are killed at temperatures above freezing point and become comatose from cold before there is danger of death. Self-protective responses and development are inhibited when their surroundings are comparatively warm. Many insects would be incapable both of completing their life-cycles and of avoiding extermination unless the environmental temperatures were well above zero for a considerable period each

year. Such insects become rapidly acclimatized by brief exposure to a slightly colder environment. Thus, the chill-coma temperature of cockroaches kept at 30° C. is 7·5° C., but when individuals from the same stock are kept at 15° C. for as little as 24 hr. they afterwards move actively at 2° C. Similarly, the alarm reaction of larvæ of the yellow fever mosquito (*Aedes aegypti*) can be elicited at lower temperatures after exposing them for 24 hr. to colder water. The processes of acclimatization in insects are rapidly reversible, and their underlying mechanism remains a mystery. Insects of a second large group survive exposure to temperatures below freezing point provided that they remain supercooled. Crystallization of ice in their tissues is fatal to them. The degree to which they can be supercooled depends on the duration of exposure to that temperature. It also depends on the water content of the tissues and of the material in the gut. No material exists more water-proof than insect cuticle. Nevertheless, when a drop of moisture freezes on the outer surface of a supercooled insect the tissue fluids beneath the cuticle are instantly inoculated with ice and freeze. Recently, Asahina and Aoki[1] reported that certain moth larvæ survived freezing in liquid air under conditions in which the greater part of their body-water was converted into crystals of ice situated outside the individual cells composing the tissues. Dr. Mellanby pointed out that, from an ecological point of view, liquid air is not an important habitat. On the other hand, witnesses of the advent of spring in the Arctic describe the abundance of active insects before the snow has melted sufficiently to uncover sheltered spots with warmer micro-climates[2]. The third group of insects which survive freezing at low temperatures may, therefore, be larger and more important than hitherto supposed.

Rome and Cape Cod are at approximately the same latitude (42°). It was therefore surprising to learn from Dr. J. W. Kanwisher that temperatures between − 15° and − 30° C. are frequently recorded on the shore at Woods Hole in winter. The intertidal molluscs are in open air for 6 hr. or more around low tide twice each day. When not covered by sea water the internal temperatures of oysters, mussels and periwinkles are almost identical with the atmospheric temperature. At − 15° C., 60–85 per cent of the water in the bodies of these molluscs was frozen. Nevertheless, intact molluscs of the same species recovered fully when thawed after freezing to the same extent. Histological sections of tissues from the frozen animals showed remarkable distortion of the muscles and of the internal organs by ice crystals situated outside the cells which were shrunken. Within a few seconds of thawing the microscopic anatomy was normal. When water crystallized as ice the tissues were exposed to increased salinity, reaching a maximum at − 20° C. The tissues of the intertidal molluscs became isosmotic with concentrated solutions of salt to which they were exposed experimentally. A change in temperature of 10 deg. C. altered their oxygen consumption by a factor of 2 in the range above 0° C. and by 50 below 0° C. An increase in the salt concentration above zero equivalent to the increased salinity of partially frozen sea water cooled by 10 deg. C. reduced the oxygen consumption of the molluscs by a factor between 10 and 20. The striking alteration in the $Q_{10}(O_2)$ of the frozen animals cannot, therefore, be entirely explained by the combined effects of raised salinity and reduced temperature. Decreased diffusion of gases and increased viscosity of residual tissue fluids may also be involved. Many molluscs are immobile at 0° C. Some teleost fish, on the other hand, swim in Arctic waters at a temperature which remains steadily at −1·7° C. at the bottom and fluctuates near the surface between −1·7° C. below the ice in winter and +7° C. after the ice has melted in summer. The freezing point of blood from teleosts in temperate waters is between −0·5° C. and − 0·8° C. The freezing point of plasma from five species of deep-water Arctic fish was between − 0·9° and − 1·0° C. These fish were actively swimming with their tissue fluids in the supercooled state. They were in no danger of being seeded with ice crystals in their own ecological niche, but when brought near the surface on which ice was floating they soon froze throughout and perished. By contrast, the plasma of the Arctic surface fish froze at − 0·8° C. in summer and at − 1·5° C. in winter. The osmotic concentration of their body fluids had doubled before winter set in, so that they became supercooled by less than 0·4 deg. C. The risk of freezing was thus reduced when ice formed nearby. Dr. Kanwisher's demonstration that animals can retain full activity in the supercooled state has introduced a new conception into low-temperature ecology.

The practice of storing semen in the frozen state at low temperatures is no longer new. Nine years have passed since the report was published that glycerol protected avian spermatozoa against damage during freezing and thawing[3]. Seven years ago the possibility of banking glycerol-treated frozen bull semen was discussed at the British Association. Since then the technique has been adopted at every major cattle-breeding centre where artificial insemination is practised. A review by Dr. C. Polge, the leading authority on the subject, was therefore welcome. He said that it was essential to restore the motility of a high proportion of spermatozoa in semen to be inseminated after freezing to and storage at low temperatures. Dilution to contain a suitable concentration of glycerol and a slow rate of cooling are the two procedures vital for preserving sperm from most mammals. Human spermatozoa are exceptional. Some survive when ampoules containing untreated semen are frozen rapidly by immersion in liquid nitrogen at − 196° C. Spermatozoa of the bull and other mammals are killed by rapid cooling either above or below zero. Physico-chemical changes in the cell membrane may be responsible. Ultra-rapid cooling might cause instantaneous formation of minute intra- and extra-cellular ice crystals. Slow cooling results in growth outside the cells of larger crystals which might be less destructive; simultaneously, however, the concentration of salts rises and the spermatozoa are pickled in strong brine. Glycerol buffers the salts during freezing and permits slow cooling to avoid thermal shock. Other neutral solutes act similarly, but glycerol is unique in its lack of toxicity in high concentrations at low temperatures. The optimum initial concentration of glycerol is approximately 10 per cent for protecting bull spermatozoa, but varies for sperm of other species according to their electrolyte sensitivity and is 0–5 per cent in human and 30 per cent in rabbit semen. The motility of glycerol-treated spermatozoa of ram, boar and rabbit is high after freezing, but the fertility of the thawed semen, assessed by artificial insemination, has so far been reduced or abolished

in these species. Human pregnancies have been reported in the United States following insemination of glycerol-treated frozen semen[4], but no critical experiment has been or is likely to be carried out in Great Britain. The fertilizing capacity of bull semen is not impaired by addition of glycerol and prolonged storage at − 79° or −196° C., as judged by the proportion of cows which become pregnant and the millions of normal calves already born as the result of its use. Semen of the 1952 Cambridge vintage, including samples from a bull now dead three years, still show little, if any, reduction of activity or fertilizing capacity. Farmers are already exploiting a wide choice of bulls not previously at their service. When results of progeny tests on young bulls are obtained, large stocks of semen from the best sires will be available locally and for export. Great improvements in cattle are anticipated throughout the world. Eventually, perhaps, the technique will be applicable to other farm animals.

A wide variety of other mammalian cells and tissues can now be frozen and stored without loss of viability in media containing glycerol at low temperatures. The story of how bone marrow came to be preserved in this way was told by Dr. D. W. H. Barnes. The starting-point was Jacobson's discovery in 1950[5] that mice could be saved from death after lethal doses of X-rays if, afterwards, fragments of infantile mouse spleen were inserted intraperitoneally. The explanations proposed were either that a chemical agent from spleen protected tissues sensitive to irradiation or that hæmopoietic cells from the implanted tissue repopulated the bone marrow, which would have been totally destroyed by the doses given. The report by Lorenz[6] that marrow from guinea pigs would protect mice of certain strains boosted the humoral theory. However, Barnes and Loutit had evidence that any treatment which killed the splenic cells also abolished their protective effect. One such treatment unlikely to harm a chemical agent was storage at + 4° or at − 15° C. Splenic tissue was therefore treated with glycerol and cooled to − 79° C. by the method used for preserving the viability of ovarian tissue. When the material was thawed and injected into mice after irradiating them with the lethal dose of 950 r., the proportion of survivors was as high as in the control group treated with fresh spleen. There was no loss in potency of tissue after 83 days at − 79° C. Rat bone marrow was then found to be effective in treating irradiated mice, and Barnes observed an early regeneration of hæmopoietic tissue. There are distinctive differences between the chromosomes of the mouse and the rat, which enabled Ford and Hamerton to demonstrate large numbers of dividing rat cells in the marrow, spleen and lymph glands of the treated mice[7]. Radiation had destroyed the ability of the animals to react against the foreign cells which had therefore colonized the damaged hæmo- and lympho-poietic tissues. These mice also accepted grafts of rat skin, which survived indefinitely and were recognized by the colour and length of the hairs. Rat bone marrow which had been stored in the frozen state gave results as good as those obtained with fresh marrow.

Hitherto, the concept of animals composed of tissues from two or more species has belonged to mythology. The modern chimæra, produced after irradiation, is a brilliant departure from conventional biology. It is gratifying that the use of low temperatures should have contributed to its recognition. Hopes can now be renewed of establishing banks of frozen but viable bone marrow and other tissues to replace deficient or disordered tissues in man.

Re-animation of dead and frozen animals and man is another concept with origins in antiquity. Claims to have witnessed or effected such resurrections have been made and refuted many times in ancient, medieval and modern times. Superficial tissues can be frozen without altering the internal temperature or endangering the life of the animal as a whole, although frostbite may cause local death and loss of extremities. Man and other mammals become anæsthetized when cooled by as little as 10–15 deg. C. Claude Bernard showed that guinea pigs recovered when rewarmed from internal temperatures of 18°, but at 16° C. breathing and heart beats stopped and they died. Cooling to approximately this degree was lethal to all non-hibernators. The demonstration by Andjus[8] that rats could be resuscitated from body temperatures between 0° and 2° C. and after 1 hr. of respiratory and circulatory arrest necessitated revision of criteria of death and opened new possibilities for therapeutics and experimentation. Dr. A. U. Smith has cooled golden hamsters until breathing and heart beats were at a standstill and then immersed them in baths at − 5° C. to reduce the deep body temperature below zero. About 75 per cent of the animals froze progressively while the deep body temperature remained at the freezing point of plasma (− 0·6° C.). At the end of an hour the proportion of water converted to ice was 90 per cent in the subcutaneous tissues, 60 per cent in the brain and 50 per cent in the entire body. Nevertheless, hamsters frozen for periods up to 1 hr. recovered fully after rewarming the whole body with diathermy and giving artificial respiration. Frost-bite only occurred if the extremities had been bent in the frozen state. About 25 per cent of the hamsters became supercooled and were resuscitated completely from body temperatures as low as −5·0° C. Those which crystallized spontaneously after supercooling to temperatures below − 3·0° C. did not revive. Difficulties arose when rabbits and small primates (*Galago crassicaudatus*) were frozen at and rewarmed from body temperatures below 0° C. Heart beats, breathing and spontaneous movements were usually regained after thawing, but the animals died a few hours later, showing that present techniques are inadequate. Experiments on freezing isolated uteri and hearts suggest that eventually it may be possible to perfuse the whole mammal with glycerol and revive it after freezing at temperatures as low as those used for storing isolated cells and tissues. Much remains to be done before these results could be applied to man.

It was remarkable that this symposium should have assembled so much information not discussed at recent conferences on low-temperature biology[9,10]. Many new problems were also posed, some of which, it is hoped, will be solved by scientists of other disciplines and of the rising generation who had gathered at the British Association's meeting in Glasgow.

AUDREY U. SMITH

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APPLICATIONS OF CRYSTALLOGRAPHY TO CHEMISTRY

SECTION B (Chemistry) of the British Association on August 29 devoted a morning session of the annual meeting held in Glasgow to a discussion of the chemical applications of modern crystallography. The meeting was well attended and three papers were presented dealing, respectively, with applications to organic chemistry, inorganic chemistry, and with the packing of molecules in crystals.

In his paper on "The Measurement of Molecules by X-Rays" Prof. J. Monteath Robertson first outlined the classical methods and concepts of organic chemistry by means of which the relative positions in space of all the atoms in many hundreds of thousands of organic molecules have been determined, starting with the fundamental ideas of Kekulé and Archibald Scott Couper of Kirkintilloch. In contrast, the X-ray crystallographic method, developed during the past forty-six years, proceeds by direct measurement and calculation from the diffraction pattern. Kekulé's formula for benzene was shown and compared with the latest electron density section evaluated in the plane of the molecule by Prof. E. G. Cox[1]. This shows a time average of the distribution of electrons, and the atoms stand out as peaks on the contoured diagram. The main cause of the blurring of the atoms in this diagram is the fairly large amplitude of thermal movement that they possess in the crystal even at −3° C., the temperature at which the measurements were made. The motion is mainly an oscillation of the whole molecule of amplitude 7–9 deg. about the six-fold axis.

Similar sections of the naphthalene and anthracene molecules were shown, based on a very complete evaluation of the X-ray intensity data[2]. These crystals have higher melting points than benzene, and the thermal movements of the atoms, although still large, are less than in benzene. The definition of the atoms is now so good that the bond-lengths can be estimated to within a few thousandths of an angstrom.

Further elaborate refinements have recently been carried out on these molecules to try to obtain evidence regarding the distribution of the electrons that form the chemical bonds between the atoms. Briefly, the method consists of calculating the theoretical distribution of electrons in an isolated or non-bonded carbon atom. The thermal movement, which smears out the electron distribution, is then carefully calculated from the X-ray intensities. The theoretical atoms, each with its proper thermal movement but non-bonded, are then subtracted from the experimentally observed electron distributions. Any residual electron density may then be attributed to the effect of the chemical bonds. At this limit of refinement the accuracy of the observations (the measured intensities) is not sufficient to yield really conclusive results. But the results which have recently been obtained by D. W. J. Cruickshank[3] provide fairly definite experimental evidence for a ridge of residual density running around the aromatic rings and along the directions of the chemical bonds. This is in striking agreement with chemical theory for aromatic systems.

In addition to this work on simple molecules, a second and most important application of the X-ray diffraction method is in dealing with new, chemically unknown or incompletely known structures, where the object of the work is not so much extreme accuracy as just to locate the atoms in order to discover the structural formula. Serious problems of phase determination now arise, and the most generally successful method has been the use of a single atom or small group of atoms somewhat heavier, or containing more electrons, than the atoms of the unknown carbon–hydrogen skeleton of the molecule. The method was illustrated in a simple form in the structure determination of nickel phthalocyanine[4].

With the compounds of organic chemistry it is generally fairly easy to insert a marker atom without unduly disturbing the structure. Halogen atoms may often be attached, or if the compound is an acid then heavy metal salts may be prepared. It is probably true to say that this general method has been utilized, in some form or another, in the great majority of X-ray structure determinations of complex organic compounds during the past twenty years. The list of successful determinations is now so large that it would be tedious to try to enumerate them all. The structures of many natural products in the terpene and alkaloid fields, and several important antibiotics, have been determined in this way.

To begin with, the results have generally confirmed and put on an exact metrical scale what the chemist already knew, in outline at least. But as the technique of the subject advances the solution of chemically unknown structures is becoming more feasible. Already some notable contributions have been made. In the terpene field the structures of longifolene[5], lanostenol[6], and oleanolic acid[7] have been solved. The structure of the alkaloid isocryptopleurine was solved by Fridrichsons and Mathieson[8] without any chemical data except the empirical formula, and the tropolone derivative nootkatin[9] was recently solved in Glasgow.

Much the most striking recent result in this field, however, is the solution of the structure of vitamin B_{12} by Dorothy Crowfoot Hodgkin, John White and their many collaborators[10]. When this work began in 1948 there was no information about the chemical nature of the vitamin. But during the next eight years the chemical structure of various groups within the molecule was determined. A nucleotide-like group consisting of a benziminazole ribose phosphate, a propanolamine residue and various amide groups were discovered. There was also a cyanide group and, most important, a large fragment containing cobalt which it was suspected might be a porphyrin-like nucleus. There was, however, no very direct chemical evidence as to how all these groups might be linked together.

The presence of the cobalt-containing fragment and the possibility of a nearly planar porphyrin group (the crystals are strongly pleochroic) stimulated the X-ray work. If a structure is not too complex, the presence of a single atom like cobalt, favourably situated, may sometimes lead to a very complete

structure determination. But vitamin B_{12} is vastly more complex than the compounds we have been considering, and with phases based on the cobalt contribution the density maps were very blurred and confused.

At this stage help was obtained from a study of the various other compounds that had been prepared by the chemists. In addition to the wet and air-dried vitamin, two other compounds proved to be most useful. These were the seleno-cyanate derivative, $B_{12}SeCN$, with a second heavy atom, and most important of all, a hexacarboxylic acid obtained by degradation (alkaline hydrolysis) which contained the porphyrin nucleus and its side chains. This crystalline fragment, obtained by Todd and his co-workers, was much less complex than the full vitamin ($C_{46}H_{58}O_{13}N_6CoCl$ instead of $C_{63}H_{88}O_{14}N_{14}PCo$, but these formulæ were not established until later). Even this fragment, however, still represented an excessively complex structure determination.

The general method of attack was now to pursue all these structure determinations simultaneously. When some atoms or groups of atoms became fairly definite, and were confirmed by their appearance in the maps obtained from the other derivatives, then those atoms were inserted in the phasing calculations in gradually increasing numbers, which resulted in diagrams of increasing clarity and definition. The process of gradually deducing the structure by successive approximations is not, however, such a straightforward and easy task as it may sound. If a false atom is inserted in the phasing calculations it will appear in the place where it is put. But if false it will generally cause a peak of less than the expected height and be more poorly defined than would be the case with a genuine atom correctly placed. The work requires much judgment and intuition, and a continual process of checking against what is plausible on chemical grounds.

Another difficulty in this work, of course, is the tremendous amount of numerical calculation required. Each stage involves the evaluation of a triple Fourier series of several thousand terms at many tens of thousands of points throughout the unit cell. It is essentially a job for the large electronic computers; and the later stages of the work owed much to Dr. Trueblood and the computer SWAC at Los Angeles.

In the latest work the positions of all the atoms in the molecule and of about 12 out of an estimated 24 molecules of water in the wet crystal have been ascertained. This very precise and beautifully determined structure should now form a basis for the better understanding of the biological activity of the vitamin, its relationship to the porphyrins and to porphobilinogen, and may also perhaps lead to its ultimate chemical synthesis.

This example was described at some length because it illustrates better than any other the enormous power of the X-ray method of analysis when applied to the solution of complex structures. When we remember the steady advances in technique which are taking place, and in particular the design of faster and more efficient electronic computers, we begin to realize what the effect of this new method on the future progress of chemistry is going to be. In determining the positions of atoms in space, which is to a large extent the basis of chemical knowledge, we have certainly moved a long way from those first fumbling steps which were taken about a century ago.

In the next paper, by Peter Pauling, on "A Rebirth of Inorganic Crystallography", a very brief review of the progress of X-ray analysis in the inorganic field was given. The early structural data, the Bohr atom and the new quantum mechanics allowed a theory of inorganic chemical bonding to be developed which extended the co-ordination theory of Werner and the covalent bond of Lewis. This went well on the way toward giving inorganic chemistry a unified theory, but it is certainly not complete. The post-war ligand field theory has had good success in explaining certain spectral phenomena and structural anomalies, but it is still not complete, as crystallographers and chemists have shown.

The impact of these theories has caused a renewed interest in inorganic chemistry and the structures of inorganic compounds. Inorganic chemistry still does not have a unified theory, though a means of thought and a direction in which to go are known, and it is certain that a complete theory will contain most important terms based on the spatial arrangement of the atoms as determined and checked by diffraction techniques.

In the last paper, on "How Molecules Fit Together", H. M. Powell said the fact that molecules fit together at all may seem remote from social significance, but an audience is itself, in a physical sense, composed of very little other than molecules fitted together, and the lecturer's every act of thought and speech involves rearrangement of his own constituent molecules. Such matters are very complex, but some help towards their understanding may come from the study of how simpler molecules pack in non-living material.

Molecules have shapes, some rigid, some flexible, determined by the distances between their linked atoms and the angles between the links. For packing purposes each atom in the atomic skeleton may be looked upon as surrounded by a negatively charged electron cloud, so that a molecule may be pictured as something like a balloon of possibly very irregular shape. Chemical reactions, growth, drug action, carcinogenic activity, and many other matters are bound up in various ways with molecular shape. In contrast to the liquid and gaseous stages, in which molecules behave in many ways as spheres, the crystalline pattern has the molecules in fixed positions which are determined by the manner in which their shapes can be packed. A general principle is that the molecules under the influence of their attractions tend to be closely packed, globules like piles of shot, cylinders like cigarettes or sloping like packs of books in a partly filled shelf, disks parallel like a pile of plates. More localized attractions between particular parts of neighbouring molecules, for example, strong spatially directed attractions through hydrogen in neighbouring water molecules, may act in a contrary sense, and by limiting the positions in which one may be placed relative to its neighbours, may lead to very open structures such as that of ice.

In some crystals molecules are bound, through hydrogen bonds or in other ways, to form larger aggregates. These sometimes may extend indefinitely in one or two dimensions, when the problem of packing them becomes that of arranging extended cylinders, ribbons, flat sheets, corrugated sheets, or related forms. If the aggregate extends indefinitely in three dimensions it constitutes the whole crystal and there is no further packing problem so far as that structure itself is concerned.

Molecular structures of great geometrical complexity may be designed, and sometimes a close packing is impossible. Sometimes, molecules of two or more different kinds, if they are present in some suitable mixture, will together form a crystalline molecular compound. The manner in which molecules of different kinds thus fit together in regular or irregular arrays gives further information on the general principles of molecular packing.

Among the molecular packings in crystals, which were illustrated, were those in which similar or different shapes adjoin, and some where one shape encloses another. Others may interpenetrate like the links of a chain or become interlocked in a manner analogous to that used in a zip fastener. The dimensions and regularity of patterns formed by adjoining or enclosing molecules of two kinds can be related to the dimensions of the participating molecules.

J. Monteath Robertson

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NEWS and VIEWS

Botany at the Imperial College of Science and Technology: Prof. W. O. James, F.R.S.

The news of the appointment of Dr. W. O. James to the chair of botany and headship of the Department at the Imperial College of Science and Technology will be received with satisfaction by British botanists. He succeeds Prof. P. H. Gregory, who has been appointed head of the Department of Plant Pathology, Rothamsted Experimental Station (*Nature*, **181**, 158; 1958; **172**, 838; 1953). Dr. James began his botanical career as a student under Prof. Walter Stiles at what was then University College, Reading, and after obtaining a first-class honours degree in the University of London, for which Reading students then sat, he worked on problems of photosynthesis with the late Dr. F. F. Blackman at Cambridge. This was followed by a period at Rothamsted, where he examined the effect of potassium on carbohydrate metabolism. In 1928, Dr. James joined the staff of the Botany Department at Oxford, then under the direction of the late Prof. (afterwards Sir Arthur) Tansley. At Oxford, Dr. James has built up a very flourishing school of research in plant physiology, attention having been chiefly directed to the mechanism of plant respiration, for the elucidation of which, and the establishment of its essential identity with that in animal tissues, the work of James and his pupils has been largely responsible. This work has been described in numerous papers which have appeared mainly in the *Proceedings of the Royal Society*, the *New Phytologist* and the *Biochemical Journal*. During the Second World War, as part of the Oxford Medicinal Plants Scheme, Dr. James devoted much time to the study of alkaloid metabolism and its relation to respiration. As well as his well-known monograph on "Plant Respiration", published in 1953, Dr. James is the author (jointly with Prof. A. R. Clapham) of "The Biology of Flowers", published in 1935, in which the authors dealt with the forms of flowers of a representative selection of species and their relations to insect visitors. His "Introduction to Plant Physiology" is also well known. Since 1932 Dr. James has been one of the editors of the *New Phytologist*.

Museum of Natural History, Washington: Dr. A. C. Smith

Dr. A. C. Smith has been appointed director of the Museum of Natural History of the United States National Museum. He has been programme director for systematic biology with the National Science Foundation. Of special interest in this appointment is that, prior to his affiliation with the National Science Foundation, Dr. Smith was, for eight years, curator of the Division of Phanerogams in the Smithsonian Institution's Department of Botany. He now returns to the Institution to direct the Bureau, which includes the Departments of Anthropology, Zoology, Botany and Geology. In addition to directing the activities of the Museum of Natural History, Dr. Smith will continue his work in phanerogams, a research area of paramount importance. To date, Dr. Smith has published some 135 technical papers concerning this phase of botanical research. He received his Ph.D. in 1933 from Columbia University in New York City. Thereafter, he was associated with the New York Botanical Garden and the Arnold Arboretum of Harvard University. In addition to study and research in England, France, Switzerland, Germany, Holland and the Scandinavian countries, he has participated in several botanical expeditions to Colombia, Peru, Brazil, Fiji, British Guiana, and the Lesser Antilles.

Supreme Headquarters Allied Powers Europe: Dr. J. W. Abrams

Dr. John W. Abrams, of Ottawa, who is a member of the Canadian Defence Research Board and scientific adviser to the Chief of the Air Staff since 1954, has been loaned to Supreme Headquarters Allied Powers Europe (SHAPE) to undertake important duties in connexion with the scientific analysis and planning of Western European air defences. Succeeding him at R.C.A.F. Headquarters is Dr. J. C. Arnell, of Ottawa, the Board's director of plans. Both Dr. Abrams and Dr. Arnell assumed their new duties on September 1. General Lauris Norstad, supreme allied commander, Europe, requested Dr. Abrams's services because of his wide experience in the field of operational research and, in particular, with the various scientific problems relative to air defence systems. He played a prominent part in investigating the scientific aspects associated with the planning of Canada's air defence effort.

Research on High-energy Nuclear Particles

The U.S. National Science Foundation has made a grant of 450,000 dollars to the University of Chicago for high-energy nuclear particle research with

balloons. The team will be led by Dr. Marcel Stein, of the University. The balloon flights will be part of a project investigating cosmic rays in the region of 10,000 BeV. Large stacks of photographic sheet emulsion will be sent by unmanned 'Skyhook' balloons to an altitude of 120,000 ft. for a 24-hr. period. Pressurized, light-tight gondolas attached to the balloon will contain the stacks, each of which measures approximately 2 ft. × 1½ ft. × 1 ft. This is about five times the size of comparable film stacks which have previously been sent up. A particle from outer space, such as a proton, entering the stack collides with a heavy atomic nucleus in the emulsion and the resulting nuclear explosion generates a jet of very energetic particles. This jet, like a burst of particles in an accelerator, strikes other nuclear targets and produces a whole series of new nuclear reactions, all recorded on the emulsion stacks. The purpose of the study, which will continue for three years, is to investigate high-energy elementary nuclear particles which can only be observed at the high altitudes reached in balloon flights. Calculations show that the flights should yield a substantial number of collisions above 10,000 BeV. and as many as 30,000 interactions at the lower but still significant 100-BeV. energy level. Quantities to be measured by the experiment include: primary energies of nucleons, number of charged and neutral secondary particles, interaction mean free paths of secondary particles, cross-sections from primary and secondary interactions as a function of their energy, energy loss per collision of nucleons, angular and energy distribution of secondary particles as a function of primary energy, energy spectra of heavy nuclei, and break-up in the cosmic radiation.

New Round-the-World Telephone Cable

THE construction of a round-the-world Commonwealth telephone cable was considered at the Commonwealth Trade and Economic Conference in Montreal. It was envisaged that its provision would be based on capital contributions from all Commonwealth countries and that the new cable could be operated on a self-supporting financial basis. The first trans-oceanic telephone cable was opened in 1956 between the United Kingdom, Canada and the United States, and the construction of a further cable between Canada and the United Kingdom is expected to be completed in 1961. This would form the first part of the new Commonwealth link. The rest of the route considered by the experts would be as follows: the west coast of Canada would be linked with New Zealand and Australia, and from there the cable would run through the Indian Ocean connecting Malaya, India, Pakistan and Ceylon, and from these countries by way of Kenya to South Africa. By providing a link between South Africa and the United Kingdom, connexion would be made with trans-Atlantic telephone cables.

The order of construction must be a matter for further consideration by Commonwealth Governments, but it may be that the existing and proposed links from the United Kingdom to Canada would first be extended by a cable from the United Kingdom to South Africa and a cable across the Pacific to New Zealand and Australia; the links spanning the Indian Ocean, to be constructed later, would complete the girdle of the world. The conference estimated that the total length of a round-the-world telephone cable of this nature would be about 27,000 nautical miles, or nearly 30,000 nautical miles if the trans-Atlantic crossing is included. The construction cost was estimated at £88 million, including the trans-Atlantic cables.

Russian Scientific and Technological Literature

THANKS to the co-operation of many organizations in Britain and abroad, the Department of Scientific and Industrial Research Lending Library Unit now has a growing collection of recent Russian scientific and technological literature. Despite its imperfections, it is already the largest in Western Europe. Lists of what Russian literature has been received are being issued, but the demand for this literature is not very great. It is clear that owing to the linguistic difficulties something more than the traditional library methods of making known what is available is required. Suggestions for new services will be considered. Whenever translations of Russian into English become available the Unit obtains copies if the cost of the translations is less than the actual cost of making new translations. Nevertheless, these measures by themselves appear to be inadequate, so it has been decided to initiate a translating programme. The basic idea behind the programme is that financial assistance should be available where necessary to help specialist organizations to select, translate and arrange for the publication of the important Soviet scientific and technical literature in their fields. This programme is being drawn up to avoid any duplication with the translating activities in the United States, and details about it will be made known later. Further information can be obtained from the Unit at 20 Chester Terrace, London, N.W.1.

New 'Area of Outstanding Natural Beauty'

THE Minister of Housing and Local Government, Mr. Henry Brooke, has confirmed an Order made by the National Parks Commission under the National Parks and Access to the Countryside Act, 1949, which has the effect of establishing about 26 square miles of countryside in Staffordshire, including Cannock Chase, as an 'area of outstanding natural beauty'. The area extends between Brocton, Tixall, Rugeley and Gentleshaw and also includes Shoal Hill Common to the south-west of Cannock Chase. Government grants can be made at the rate of 75 per cent towards the cost of treating derelict land, tree planting and preservation, and removing disfigurements. Grants are also available towards expenditure incurred in making agreements or orders for public access to open country and in appointing wardens. Designation does not of itself provide any additional right of access to private land. This is the sixth such area to be established. The others are: the Quantock Hills and Surrey Hills in the south of England, the Northumberland coast in the north, and the Gower and Lleyn Peninsulas in Wales.

Corby Young Naturalists' Club

CORBY Young Naturalists' Club is now in its fourth year (*School Nature Study*, 53, No. 211; April 1958). It was formed first as a group of school children who liked rambles with someone who could identify and talk about the things they saw. From there it grew to a fully fledged club with meetings and a definite object to the rambles—a 'survey' of the local woods. Field work formed the basis of the Club. So many children were interested, and joined in the activities in their spare time, that it has been found necessary to work, not as a body, but in groups,

with older children acting as the leaders. After an excursion all gather together and the various records are taken and discussed. Any doubtful record is checked in the field. All records are then entered in a book to be held for future reference. During the school holidays other field work, such as counts of birds, counts of plants in a square, or series of squares, transects of areas, and similar studies are carried out. Certificates are awarded for individual field work, or individual study. 'Report forms' are also issued to all members to encourage them to record things they see. On these records the annual report is based. One evening a week during term-time a school classroom is hired and an indoor meeting arranged. This consists of a film or film-strip, or talk by experts on a variety of subjects; the subjects are chosen by a committee of children. Outings to places of interest are also arranged. These include marshes, woodland areas, a heronry, a large winter roost for rooks and a winter roost for gulls. There is a small club library, from which the children may borrow books.

Courses on Nuclear Energy in Europe

A new edition of the "Catalogue of Courses on Nuclear Energy in O.E.E.C. Countries", covering the academic session 1958–59, has recently been issued by the European Nuclear Energy Agency of the O.E.E.C. (pp. 115. Paris: European Nuclear Energy Agency, Organization for European Economic Co-operation, 1958. 2, rue André-Pascal, Paris 16). It lists details of the courses on nuclear physics, nuclear engineering and related topics to be held in universities, technical colleges and research centres, together with practical information such as the names of the instructors or professors in charge, the durations of the courses, conditions of admission, fees, and the special pieces of equipment available. The information provided is not regarded as complete, but the catalogue should enable science and engineering students who wish to acquire a better knowledge of nuclear subjects to find out what training facilities exist and where and how to apply for them. The intending student is warned in the foreword that further information concerning any particular course should be obtained directly from the institution concerned, but it would have been perhaps wise to repeat this warning boldly throughout the catalogue, because in the case of the United Kingdom institutions, for example, the information given is neither well nor systematically chosen. To university authorities and others the catalogue will also be useful as a means of comparing the programmes of instruction in the educational establishments of the numerous countries listed and as a means of assessing the extent to which nuclear energy work has developed.

National Institute of Industrial Psychology Courses

The National Institute of Industrial Psychology has issued details of courses and seminars arranged under its auspices during October 1958–May 1959. These include courses on personnel selection methods, held in three parts each of one week's duration, with two fourth parts, each of three days duration (open to those who have already attended the first three), on the selection of clerical personnel or, alternatively, of engineering apprentices. A course on the use of statistics in personnel management has been arranged jointly with the Institute of Personnel Management. A three-day course on staff reporting and merit-rating procedures, a five-day course in vocational guidance for youth employment officers and careers teachers, and two three-day seminars on psychological problems in inspection work and on personnel work in research organizations have also been arranged. Further particulars can be obtained from the Courses Secretary, National Institute of Industrial Psychology, 14 Welbeck Street, London, W.1.

Computer Symposia

The 1958 Computer Applications Symposium, organized by the Armour Research Foundation of the Illinois Institute of Technology, is to be held in Chicago on October 29 and 30. The purpose of the symposium will be to discuss computing and data processing devices from the point of view of users of such equipment. Business and management applications of computers will be stressed on the first day, engineering and scientific applications on the second day. Forms of application and further information can be obtained from M. J. Jans, Conference Secretary, Armour Research Foundation, 10 West 35th Street, Chicago 16, Illinois.

A Business Computer Symposium is to be held at Olympia, London, during December 1–3, in conjunction with the Electronic Computer Exhibition being held during November 28–December 4. An opening address is to be given by the Earl of Halsbury, managing director of the National Research Development Corporation, at whose instigation the Exhibition and Symposium have been organized jointly by the Electronic Engineering Association and the Office Appliance and Business Equipment Trades Association. The papers to be presented will cover practical experience of the use of computers for pay-rolls, production planning and management, file maintenance, public utility accounting, farm accounting, banking, insurance, stores control, research and design. Enrolment forms and further information can be obtained from the Electronic Computer Exhibition, 11/13 Dowgate Hill, London, E.C.4.

Announcements

Sir Alexander Fleck has been appointed chairman of the Scientific Advisory Council of the Ministry of Power, which was set up in 1948 to advise the Minister on the scientific aspects of his statutory duties. Its first chairman was Sir Alfred Egerton, followed in 1954 by Sir George Thomson.

The Institute of Personnel Management is holding its 1958 National Conference during October 10–12 at Harrogate, on the theme "The Individual and the Organization in Modern Society". Further information can be obtained from Miss E. M. Harris, Institute of Personnel Management, 80 Fetter Lane, London, E.C.4.

A symposium on "The Tools of Biological Research" is to be held in the Physiology Theatre, Guy's Hospital, London, S.E.1, on October 10 and 11, when the opening address will be delivered by Sir Cyril Hinshelwood. Further information can be obtained from Mr. H. J. B. Atkins, Department of Surgery, Guy's Hospital.

Erratum. In the article "Partition of Proteins in Liquid Polymer–Polymer Two-Phase Systems", by Dr. Per-Åke Albertsson, in *Nature* of September 13, p. 709, 'System *G*' should read 'System *C*' in: (1) Table 1, column 3; (2) the legend to Fig. 2; (3) p. 710, column 2, eighth line from the bottom.

MODERN EDUCATIONAL TREATMENT OF DEAFNESS

UNTIL recently there has been less investigation of hearing and of auditory perception than of vision and visual perception. Modern technological advances have made it possible for workers in a number of fields to gain much new knowledge about hearing. The International Congress on the Modern Educational Treatment of Deafness, which was held in the University of Manchester during July 15–23, was attended by more than 1,000 members from forty-one countries and was the largest of its kind ever to be held. It was distinguished also for its comprehensive and eclectic character. Papers on recent advances in the physiology of hearing, the pathology and causation of deafness in childhood, experimental phonetics, psycho-acoustics and audiology and the educational treatment of deafness attracted a 90 per cent attendance throughout the duration of the Congress. Audiences that included leading medical consultants and authorities on electro-acoustics and many outstanding educators of the deaf from almost all parts of the world followed papers and demonstrations of work in each others' fields with the closest attention.

The view was frequently expressed and evidently widely accepted that new scientific data about hearing are increasingly important and indeed basic to the treatment of deafness in childhood, and that the teamwork of experts in all relevant fields which is already in operation in certain centres constitutes a necessary practical approach.

Dr. Hallowell Davis, so long well-known for outstanding experimental studies in audiology at Harvard University and the Central Institute for the Deaf, St. Louis, reported that the electron microscope has now revealed many details of structure of the sense organ of the ear. The 'hairs' on the sensory hair cells of the cochlea now stand out clearly. There are considerable and probably important structural differences between the internal and external hair cells. An alternating-current output—the familiar cochlea microphonic—comes from the external hair cells, while a direct current comes from the internal hair cells. The internal hair cells seem to resist the action of poisonous drugs and the mechanical stresses of high-intensity sound more successfully than the external hair cells, which appear to be more sensitive, more specialized and relatively recent in origin. The external hair cells make possible the detection of extremely faint sounds. Small differences in the times of arrival of individual sound waves at our two ears are detected by the external hair cells and the fibres connected to them and are one basis for our localization of the source of sound in the external world.

Microchemical studies of the fluids of the inner ear show that the endolymph within the membranous labyrinth is a tissue fluid of unique chemical composition. It has a high concentration of sodium. It has not yet proved possible to relate the possible physiological and neurological abnormalities that may be represented by recruitment and by Ménière's disease to the new physiological findings of chemistry and electro-physiology. An unexpected finding has been the demonstration of a bundle of nerve fibres that runs from the brain stem to the sense organs. Galambos has demonstrated that appropriate stimulation of this pathway (in cats under laboratory conditions) reduces the sensitivity of the ear.

Prof. Ormerod (Institute of Laryngology and Otology, University of London), in a paper on the pathology of congenital deafness, defined deafness in the very young child as due to failure to develop of some part of the auditory mechanism, or to a degeneration or a disturbance of some part, already developed, of this system. The factor interfering with the development of the ear must be operative during the first half of the period of gestation. There may be complete failure to develop of either acoustic or static parts of the internal ear. The basal coil of the cochlea may take shape but not the middle and apical turns. In the commoner form of congenital change the bony labyrinth is perfectly formed, the membranous and sensory vestibular organ is fully developed and functioning, but in the cochlea the scala media is usually collapsed and the organ of Corti is represented by a small heap of undifferentiated cells. Not all the children who suffer from congenital deafness are totally deaf, but as shown by Whetnall, the great majority have some islands of hearing and may be described as sub-totally deaf. (A similar finding has been reported from Manchester.)

Mr. Harrison, in a paper on the etiology of deafness in childhood, and Dr. Watson, dealing with factors affecting the successful use of hearing aids by deaf children, reported some results of a collaborative project for which a joint team of senior members of the staffs of the Manchester Department of Education of the Deaf and of Otolaryngology have been responsible, for a number of years, with the support of the Medical Research Council. In certain cases of deafness in childhood, for example, of malformation of the external and middle ear, capacity to hear had been improved after operative treatment, by contrast with cases of deafness associated with a history of kernicterus, which are not amenable to treatment. Familial perceptive deafness was characterized by a flattish type of audiogram, with hearing losses of about 80 decibels and by pure-tone determinations indicative of severe high-tone loss.

Dr. Goodhill (Los Angeles), in a paper given by means of a sound-recording, described recent surgical advances involving many procedures for reconstituting the middle ear, in children as well as in adults, with the view of enabling transmission of sound energy to the cochlea.

Results of systematic research on the capacity of children to benefit from the use of hearing aids were reported in several papers. Prolonged studies of groups of children in different categories of auditory impairment were stated by Dr. Watson and by Dr. Hudgins (Clarke School for the Deaf, Massachusetts) to have shown that no deduction about capacity to hear speech can be made from the pure-tone audiogram alone. In the joint investigation at the University of Manchester it had been demonstrated that some cases of perceptive deafness and most cases of conductive deafness could discriminate speech.

As regards profoundly deaf children, a study of fifty pupils of the Clarke School who were profoundly deaf, over a five-year period had shown that their speech perception scores, when tested in the condition of listening with a hearing aid, and looking, were significantly higher than in the condition of lip-reading alone, and that the scores consistently improved in both conditions.

Prof. Bordley and Prof. Hardy (Baltimore) reported that since the Hearing and Speech Center of the Johns Hopkins Medical Institutions was established in 1947, intensive medical and neurological studies of children had led to a concept of hearing problems as no longer centred principally in the ear and naso-pharynx, nor in the family background. Approximately 70 per cent of the pre-school age children referred to the Hearing and Speech Center gave evidence of central disorders of trauma or mal-development at the level of the brain, with or without peripheral involvement.

A valuable paper on hearing aids was given by Dr. Littler (Medical Research Council, London), who described the peculiar problems that arise when attempts are made to equalize frequency response of the whole aid, with its three elements of microphone, amplifier and reproducer, by the combination of selective response in two different bands of sensitivity of microphone and reproducer.

Mr. Fant (Stockholm), discussing the acoustics of speech, described intensive studies of vowel articulation which have resulted in a three-parameter specification of the vocal tract configuration. He demonstrated speech that was artificially produced. This paper was appropriately followed, at a later session, by one in which Mr. John (Department of Education of the Deaf, Manchester) reported experiments that have shown the detrimental effects of reverberation and noise in rooms on the intelligibility with which speech can be heard through hearing aids and the great deterioration in scores that accompanies increase in distance of speaker from microphone. The acoustics of rooms, in which the amount of reverberation that is present is completely tolerable to listeners with unimpaired binaural hearing, can offer the greatest difficulty to users of hearing aids. Reference was made to spectrographic studies that demonstrate the distortions of vowel and consonant formants that take place. Mr. Thomas, who has collaborated with Mr. John, read a paper on the application of their findings in architectural acoustics to the design and construction of schools for the deaf and the acoustical treatment of new schools, as now practised on their advice in many parts of the United Kingdom.

Closed-circuit television broadcasts were given during two sessions. The first was introduced by Dr. Irene Ewing, who described how the diagnostic practices of the Manchester department had grown out of her own direct and original study of the highly selective reactions to sounds made by normal very young children. Under her direction, screening tests of hearing suitable for children from four months to five years were given by health visitors from the public health departments of the City of Manchester and Caernarvonshire (in Welsh), to whom with some thousand others in many parts of the United Kingdom, she had given special training. Parent guidance and the results of home training, including the use of hearing aids, with children aged from eight months to five years, were shown by Dr. Ian Taylor and Mrs. Beaman. In a paper on this subject, Prof. Huizing (Groningen) gave results of experiments that demonstrated how a home-trained child, wearing a hearing aid from the age of sixteen months, achieved a more rapid growth of expressive language than children who had audiological treatment and conventional oral training from their fourth birthdays onwards. Prof. Huizing stressed the importance of auditory feed-back in the linguistic development of deaf children, that is, of enabling them to hear their own voices, and so far as possible their own speech.

In a short article it is possible only to mention a few of the many excellent contributions made by teachers of the deaf serving on the staffs of schools, and educational psychologists. Among the most notable was a demonstration, introduced by Father van Uden, of some methods and results of training profoundly deaf children to respond to the sound of speech and music, when very loudly reproduced. Some pupils of the Institute for the Deaf, Sint Michielsgestel, aged 11–12 years, all of whom were profoundly or sub-totally deaf, were given a lesson in the course of which they were seen composing 'play-songs' or simple melodies to which they afterwards danced. Then a similarly constituted group of girls aged 13–18 years showed their notable capacity to dance ballet to music reproduced from a battery of specially designed loud-speakers, at an average peak sound-level of 95 decibels above 0·0002 dynes per sq. centimetre. The late Dr. Becking with Father van Uden made experiments that led them to conclude that profoundly deaf children can learn to perceive feelings of vibration in the body, in response to abnormally loud sound and that these feelings are an introduction to, and an enrichment of, sound-perception in the ears, the basic principle being a feed-back between sound-perception and bodily movements.

Equally significant, in a different way, was a demonstration by closed-circuit television in which severely, profoundly and sub-totally deaf pupils from the Nursery-Infant Branch of the Royal Schools for the Deaf, Manchester, aged 7–8 years, under Miss Goldsack, showed remarkable spontaneity and capacity to express themselves verbally, with a standard of general knowledge that would have done credit to a better than average class of unhandicapped pupils. They were taught in a classroom wired for induction, which enables them to move about freely without losing the teacher's voice. Five of them had had home training. Demonstrations were given by older boys and girls, using similar equipment, from the Royal Cross Schools, Preston, and the Braidwood Schools, Birmingham. Dr. Schmaehl (Dortmund) and Mr. Mikaelian (Moscow) reported extensive use of inductance systems in their countries.

Attention was frequently directed to problems in the social development of deaf children. Miss Numbers (Clarke School, Massachusetts) and Mr. Askew, principal of the English Grammar School for the Deaf, gave details of the wide ranges of occupations that are being followed by well-educated ex-pupils of good ability. Dr. Buchli, director, Royal Institute for the Deaf, Groningen, stated that he had found that children who could not be day-pupils developed more normally when living with foster-parents than in a residential special school. Dr. Murphy (Melbourne) and Dr. Silverman, director, Central Institute for the Deaf, St. Louis, referred to deaf men and women who seek social life among others who are

similarly handicapped. Dr. O'Connor, superintendent, Lexington School for the Deaf, reported use of tests and procedures for evaluating the capacity of boys and girls handicapped by deafness to benefit from attendance at ordinary schools. Mrs. Miller (Clarke School) described some methods of social training for senior pupils. An important paper by Prof. Lewis (University of Nottingham) on language perception and reasoning was supported by one from Dr. Morosova (Moscow), in which she described capacity to use words in thinking as a leading determinant of cognitive activity. Several speakers, including the writer of this article, discussed the achievement of this capacity as a factor in the social development of deaf children, and the deviation of their mental growth from normal when it is begun later than among children who are not deaf.

A. W. G. Ewing

NATURE AND GENETICAL CONTROL OF RESISTANCE IN LIVING ORGANISMS

THE nature and genetical control of resistance in living organisms was considered at a joint session on September 1 of Sections *D* and *K* (Zoology and Botany) of the British Association at the Glasgow meeting. The topics were disease resistance in plants and animals, insecticide resistance in mosquitoes and drug resistance in bacteria.

In the opening paper Dr. P. R. Day (John Innes Horticultural Institution) outlined the types of genetical control of resistance to pathogens in higher plants. These range from the single, usually dominant, gene through complementary action of two or more genes to polygenic control. There is a similar pattern in the genetic control of virulence in fungal pathogens selected by cultivation of resistant crop varieties. At the single-gene level this has led to the suggestion of a gene-for-gene relationship whereby the outcome of host infection by a pathogen depends on the alleles present at complementary loci in both organisms. A resistance gene in the host is only effective if there is a corresponding avirulence gene in the pathogen. An example of how the gene-for-gene concept may be applied comes from work at the University of Wisconsin on the inheritance of avirulence among a group of strains of *Venturia inaequalis* (apple scab) on seven apple varieties. The genes for avirulence were identified and separated in recombinant stocks. From the results of inoculations it was possible to estimate how many genes for resistance were present in each apple variety and which genes for resistance were common to two or more varieties. The interaction of gene products of the host and pathogen enables the host to recognize that the pathogen is avirulent. This recognition triggers secondary reactions which kill or limit the pathogen growth. Mutants of *Cladosporium fulvum* (tomato leaf mould) and *Melampsora lini* (flax rust) can be obtained which presumably avoid the recognition reaction and are thus virulent on a particular host resistant to the original strains.

Miss C. E. Jackson (Ross Institute of Tropical Hygiene) described the appearance of resistance to chlorinated hydrocarbon insecticides (dieldrin, BHC and DDT) following their use to control natural populations of malaria-carrying anopheline mosquitoes. Laboratory studies showed that resistance to dieldrin in a strain of *Anopheles gambiae* from Northern Nigeria was some 800 times higher than that of wild-type insects of that species and was controlled by a partially dominant single gene. A discriminating dose of the insecticide enables hybrids to be distinguished from the homozygous resistant parent. DDT resistance in *A. sundaicus* from Java was controlled by a single recessive gene. In laboratory experiments it was possible to raise the level of tolerance of mosquitoes to insecticides by exposures to sublethal doses during twenty generations of larval selection. The level of tolerance achieved was never more than seven times that of the original stock and this fell when larval treatments were stopped. This 'vigour tolerance' may be associated with inversions found in the chromosomes of treated stocks. The importance of this problem is shown by the fact that resistance to the insecticides dieldrin, BHC and DDT and its analogues is now found in eleven countries in six species of mosquito. No example of simultaneous resistance to DDT and dieldrin or DDT and BHC has yet been proved.

Dr. Mary Barber (Postgraduate Medical School, London) described three types of drug resistance found in bacteria: drug tolerance, drug dependence and drug destruction. The first two types of resistance generally arise by mutation. Drug destruction is often due to formation of an adaptive enzyme such as penicillinase in some penicillin-resistant bacteria. The initiation of enzyme production by strains previously producing none probably results from mutation. The genetic transfer of drug resistance between cells can be accomplished in the laboratory by deoxyribonucleic acid extracts (transformation), by a virus vector (transduction) and by direct transfer of a bacterial chromosome, or parts of it, when cells are in contact (conjugation). The extent of these processes in Nature is unknown, although favourable conditions exist in the human flora or in the soil. The accumulation of drug-resistant strains of *Mycobacterium tuberculosis* and *Staphylococcus aureus* in hospitals has become a major problem. A study of penicillin-resistant *S. aureus* carried in the noses of pupil midwives in a London hospital showed a rise from 24 per cent carrying such strains during their first two weeks in the hospital to 60 per cent after three months. Of the permanent hospital staff 90 per cent carried resistant *S. aureus*.

The most successful approach to the control of both bacteria and mosquitoes is likely to be the simultaneous use of two or more drugs or insecticides. This makes it less likely that resistant mutants will develop. Plant breeders also try to release new disease-resistant crop varieties having two or more different resistance genes for the same reason.

Dr. A. Kleczkowski (Rothamsted Experimental Station) surveyed resistance to virus diseases in animals and plants. Resistance in plants is in a special category because of the absence of any immunity reaction comparable to the antibodies of the γ-globulin fraction of animal serum proteins.

Plant resistance may be due to failure of symptom expression, to interference, whereby infection with a mild strain protects against infection with a more virulent related virus, or by resistance mechanisms which result in localized death of host tissue preventing virus multiplication. Virus resistance may also be due to the host being inappropriate. Although antibodies may control the spread of virus from cell to cell, virus inside animal cells is not accessible to antibody and its amount is controlled by the rates of virus reproduction and inactivation. At higher temperatures the rate of inactivation tends to predominate and this has been used to cure virus diseases.

Virulent mutants of fungal and virus pathogens, drug-resistant bacteria and insecticide-resistant mosquitoes all arise as a result of high selection pressures. These pressures can be demonstrated even in entirely natural populations, resulting, for example, in the multiplicity of strains of *Phytophthora infestans* to be found on wild populations of *Solanum demissum* in Central Mexico.

Undoubtedly more is known about the genetical control of resistance than about its nature at the present time. As more is found out about both, so we shall be better able to use and control resistance.

P. R. Day

SCIENCE IN SCHOOLS

THE Glasgow meeting of the British Association emphasized the need to reveal and encourage scientific aptitudes from an early age, and, with this in view, a special programme for young people was devised.

The Scottish Branch of the Science Masters' Association arranged an exhibition, open every afternoon, in which typical class apparatus and experiments were demonstrated and explained by boys and girls from Glasgow schools. Both children and teachers took great interest in discovering how other schools carried out familiar experiments and confirmed well-known principles. At the same time the presence of one or two less-common pieces of apparatus, such as the van de Graaff generator, provided a little extra interest and removed the school-room atmosphere. Unilever, Ltd., had a display of charts, booklets and film-strips which are available for schools, and some of the material advantages of modern science were shown in interesting exhibitions of samples, charts and models provided by Imperial Chemical Industries, Ltd., and the United Kingdom Atomic Energy Authority.

Each afternoon of the meeting three special children's lectures were delivered concurrently, and on August 28 the boys and girls had the difficult choice of hearing Sir Lawrence Bragg on "How we hear and speak", Dr. H. Barnes on "Under-water Television" or Mr. A. E. Gunther on "Geology and Oil". Those with tickets for August 29, September 1 and 2 were in no less difficulty, since they had to choose from the following list: "Atomic Energy" by Dr. T. E. Allibone, "Prehistoric Animals" by Dr. W. E. Swinton, "Thunderstorms, Hail and Lightning" by Dr. B. J. Mason, "Exploring Flowers" by Dr. G. Taylor, "Man-made Fibres" by Mr. A. B. Thompson, "The Electronic Brain" by Dr. A. D. Booth, "Animal Clocks and Compasses" by Dr. J. D. Carthy, and "Man in Flight" by Wing-Commander P. D. G. Whittingham.

One of the features of the meeting was an open forum held on Saturday morning. Sir Alexander Fleck introduced the chairman, Dame Kathleen Lonsdale, and her panel of experts consisted of Dr. I. J. Faulkner, Mr. B. B. Lloyd, Dr. B. J. Mason and Dr. W. E. Swinton. The questions, sent in by schools in the west of Scotland, showed pronounced topicality with considerable emphasis on space travel and satellites, but the panel also had to deal with some searching questions on the nature of colour, world population and the possibility of extinction of the human species. Some uncertainty was evident among the panel when asked to define matter, but Dr. Swinton was in no doubt that the homing instinct in the pigeon was in no way due to any extra sense organ in the bird.

On Tuesday morning, at the invitation of Section *X*, some sixth-form boys read original papers. Mr. A. M. J. Hutchings and Mr. A. J. Nicholl, of the High School of Glasgow, dealt very authoritatively with "Plant Ecology of Heather, Moor and Bog", while Mr. Alan Nicol gave a concise account of "The Production of Special Steels", as did Mr. J. C. Natzio with "The Work of *Discovery II*". Both these speakers are scholars of Hutcheson's Boys' Grammar School, Glasgow. Mr. D. W. McCredie of Charterhouse read an excellent paper on "The Breeding Habits of the Hen Harrier in South-West Perthshire".

At a conference of affiliated societies, Mr. R. A. Stevens outlined the future policy of the British Association with regard to the Junior B.A. The plan requires the setting up of area committees (several are already in being and eight or nine are expected by the end of the year), which would work in co-operation with local societies to provide lectures, meeting places and organized excursions. In addition to supplying the needs of adult audiences, these area committees will do all they can to encourage school scientific societies to expand and to serve as a centre of scientific culture in the schools from which interest in science would emanate downwards into the junior classes and across into the arts side of the school. In order to give the fullest assistance to scientific societies in schools, the British Association is setting up a committee for the purpose of devising suitable visual aids, and local societies are being encouraged to organize federations of school societies in their areas for the pooling of resources and exchange of ideas. Such a plan, it is felt, would be of great value to the adult societies since it would lead the children towards them and ultimately provide a source of recruits.

It is recognized by the British Association that the present plan of holding the Junior B.A. meeting at the same time as that of the parent body is possible only in the circumstances existing in Glasgow, where the city schools have just started the new term. To hold such meetings either in the school holidays or in mid-term would be quite impracticable, and it is proposed that in future the junior meeting shall be held at the end (or beginning) of the school year in whatever area is acting as host that year.

Eric Walker

UNEMPLOYMENT AMONG WOMEN OVER FORTY

UNLIKE many societies where the bulk of the economic load is carried by women, Western culture in general and British society in particular have inherited a tradition that opposes the gainful employment of women. To the employer women workers present problems different from those of men. Employers declare that if a woman is single she tends to be temporary, filling in time until she is married; and that if she is married she has domestic responsibilities which may make her unreliable as a worker. The sickness-rate is said to be higher among women, though here it is difficult to get reliable statistics. A woman is also said to age sooner than a man, though her average expectation of life is greater. The precise accuracy of these statements is not known; the fact that they are widely believed creates an employment complication of considerable significance.

Four surveys of unemployment among women over forty years of age in the Southampton area have been made by Miss A. F. Young of the University of Southampton (*Personnel Management*, 40, No. 344; June 1958). The total number of women employed in the area has risen from about 19,000 in 1951 to more than 23,000 in 1956, and total unemployment has fluctuated between 363 in 1954, and 472 in 1953, or about 2 per cent of all women in work or seeking it. Of the women unemployed, the number over forty has varied between 88 in 1951 and 204 in 1953, that is, between about a quarter and a half of the total, though in the last two surveys the proportion has been about a third. The figures show that the number of unemployed older workers remains obstinately high even when, as in 1954, unemployment was at its lowest. This leads to the assumption that a hard core of unemployment exists among those over forty.

Nearly half the number were found to be married women, with presumably a husband and a home. Of the rest the proportion of single and widowed fluctuated, but averaged a little more than 25 per cent each of the total. It was found, however, that unemployed single women over forty sometimes reached as much as 30 per cent of the whole (1954). When those over forty were analysed according to their age, it was found that more than half were fifty or older (57 per cent, in 1956); and getting a job becomes more difficult the older a woman grows.

The 'social' reason for discouraging the older woman to take gainful employment is her responsibility to dependent children; this is the reason why married women so often interrupt their employment for a number of years. The surveys disclosed that, of the 68 married and 43 widows over forty, only 19 had any dependent children and, of these, 7 were over fifty, the rest being under fifty. This was the position in 1956, and the figures for 1954 show a similar situation. It became clear that the demands of young children are not, on the whole, a complication to be contended with in the employment of women over forty.

It is often alleged that the majority of older women are not available for full-time work, either because their health is not good enough or their domestic responsibilities make it impossible or, having left industry for a number of years, they cannot face the discipline and strain of full-time work. The facts do not support this contention; nearly two-thirds of the women in two of the surveys have asked for full-time employment. Full-time work is more possible for those under fifty than later. Against this survey it was found that, of the small number of women over sixty looking for work, nearly all wanted it full-time.

Allied to the older woman's availability for work is her reason for seeking it. Three main reasons were given: that the wage would be her sole income; that she wished to supplement her own resources; or that she needed to supplement the family income. A fourth category of "other reasons" included the need for companionship, inability to break the habit of going out to work, and other non-financial causes.

From the evidence of these surveys there is some substance in the saying "too old at forty" where women are seeking gainful employment. If this notion is to be broken at all, and the reservoir of available labour exploited to the full for the increasing productivity of our society, then some positive steps seem to be necessary. Apart from extending the number of employers who adapt their works to meet the needs of women, like part-time work of various kinds, they might include propaganda about the advantage of maturity, training, and direct recruitment into domestic and catering trades where there are many vacancies and where many older women could usefully be absorbed.

SOUTHERN STARS OF HIGH VELOCITY

AS might be expected from the fact that observatories of the southern hemisphere are of comparatively recent origin and have fewer astronomers and telescopes at their disposal than those in the northern hemisphere, the data for large groups of southern stars are by no means complete. This lack of data is particularly noticeable in the determination of radial velocities, a survey of which was completed for stars brighter than magnitude 5·5. After the publication of Miss Roman's "Catalogue of High-Velocity Stars", which included stars north of Dec. −20° and brighter than visual magnitude 9·5, it was considered that a complementary list of southern high-velocity stars would be of great interest, but, owing to the lack of observational data, it will probably be many years before a survey similar to Miss Roman's will be completed. With the object of serving as a basis for future observations the present finding list, including all available information, has been published*.

As the criteria for inclusion in the list are less stringent than for Miss Roman's "Catalogue", it includes 1,315 entries—approximately twice the number in the more selective northern list. Tangential velocities were computed for all stars south

* Memoirs of the Mount Stromlo Observatory. No. 14 (Fourth Number of Vol. 3): Southern Stars of High Velocity—a finding list compiled by William Buscombe and Pamela M. Morris. Pp. 36. (Canberra: Australian National University, 1958.)

of the celestial equator for which reliable trigonometrical parallaxes, that is, not less than 0·01″, were available, and tangential and radial velocities were corrected for a solar motion component of 20 km./sec. towards R.A. 18h., Dec. +30° relative to the nearby stars; a few other necessary corrections are also referred to. Included in the list are the stars the space velocities of which, relative to the local standard of rest, exceed 60 km./sec. as well as stars of which only one velocity component, which exceeds 50 km./sec., is known. In addition, stars the uncorrected proper motion of which exceeds 0·5″ annually and for which no further information is yet available are included. Globular clusters, planetary nebulæ and *RR* Lyr. variables, which obviously belong to population II, have been omitted, and reference is made to a number of other limitations.

Of the 1,315 stars listed proper motions were available for 97 per cent, Draper spectral types for 59 per cent, reliable trigonometrical parallaxes, that is, not less than 0·01″, for 49 per cent, radial velocities for 33 per cent, and complete information for the space velocities only 18 per cent. An unexpected feature was the presence of 37 *B* stars, which are usually considered to form part of the Population I disk system in our Galaxy. It is pointed out, however, that in many cases there are large differences between the radial velocities measured from spectroscopic plates of the same star on different dates, which may indicate that some at least of these stars are undetected binaries with large ranges in velocity relative to the Sun: in nearly every case the parallax and tangential velocity component are unknown.

It is pointed out that new data concerning stars of high velocity will be available, as southern observatories have now many observational programmes under way, and reference is made to the work in progress at a number of these observatories, including the Cape, La Plata, Cordoba, Sydney, and the Yale-Columbia Southern Station, now located on Mount Stromlo, Canberra. A few new radial velocities for bright stars have been determined from Cassegrain spectra (36 A./mm.) at $H\gamma$ taken with the Mount Stromlo 30-in. reflector. Classification on the *MK* system for these and for faint stars with Newtonian spectra (90 A./mm.) from the 74-in. reflector is in progress. Forty suspected subdwarfs are included in the current observing list.

ADULT EDUCATION IN WALES

THE report of the Universities Council for Adult Education for 1956–57* is chiefly devoted to a survey of adult education in Wales.

For the purposes of extra-mural work the Principality consists of four college areas (Aberystwyth, Bangor, Cardiff and Swansea), and there are a North Wales District and a South Wales District of the Workers' Educational Association. All provision and administration of extra-mural work is carried out by the four university colleges, the areas of which differ from one another in a variety of ways. The Aberystwyth area is entire rural. The Bangor area is also rural for the most part, but it comprises in addition the industrial districts of Flintshire and Wrexham. The Cardiff and Swansea areas are predominantly urban and industrial.

The two-and-a-half million people of Wales are shared unequally between the four areas. Half of them are in the Cardiff area, while Aberystwyth serves a population of about 300,000 scattered over an area which covers almost half of the Principality.

The development of extra-mural work has also varied from area to area. After some tentative beginnings before the First World War, Aberystwyth appointed a director of extra-mural studies in 1920 and, with a small nucleus of full-time tutors, an extra-mural department came into existence. Workers' Educational Association work in the area has not been inconsiderable, but, unaffected by industry, the country folk of this area have not responded so readily to the appeal of an association of workers as have the inhabitants of the other college areas.

The course of events in the other three colleges has been quite different. A South Wales District of the Workers' Educational Association was formed in 1907. This was reconstituted as a Welsh District in 1910 and served the Principality as a whole until the establishment of a North Wales District in 1925. University teachers took a leading part as pioneers for the Association, and throughout the inter-war period the actual organization of extra-mural work in South Wales was conducted largely through the Workers' Educational Association District. Departments of extra-mural studies can scarcely be said to have existed at Cardiff and Swansea until, at the end of the Second World War, the senior staff tutor was appointed secretary of the Swansea joint committee and a tutor in charge of extra-mural classes was appointed at Cardiff. There are still no directors at these two Colleges, though such appointments are contemplated in the present quinquennium.

At Bangor the alliance between College and the Workers' Educational Association was closer still, and the extra-mural work was administered to a considerable extent through the Association's district officer.

Each responsible body has had its own particular story of successes and difficulties. The voluntary bodies in South Wales expanded their work in a spectacular way during the industrial depression of the nineteen-thirties, when a considerable amount of money from the Carnegie Trust was made available to them. In the post-war period, on the other hand, the programmes of these voluntary organizations have dwindled to little more than half their former size, while those of the extra-mural departments of Cardiff and Swansea have grown apace. A great deal of pioneer work was done by the Aberystwyth department during the war years, and although the high figures reached then have not been fully maintained in more recent years, the present volume of work is at least twice what it was before the War. In the Bangor area there has been a striking expansion of Workers' Educational Association classes since the War and an appreciable increase in the classes conducted by the extra-mural department.

* Universities Council for Adult Education. Report on the Year 1956–1957. Pp. 27. (Bristol: W. E. Salt, Hon. Secretary and Treasurer, c/o Department of Adult Education, The University, 1958.)

Aberystwyth was the first to break away from the customary restriction of extra-mural work to tutorial and sessional classes, but, since the War, Cardiff has also been conducting an appreciable number of terminal and short courses, while Swansea has recently embarked upon a considerable programme of short extension courses. Apart from the addition of a few short extension courses, Bangor still limits itself to tutorial and sessional courses.

The Adult Education College established at Harlech in 1927 had thirty-nine students in residence for the session 1956–57, and fourteen others attended for a term or two. Almost all the sessional students were drawn from within the Principality, and about half of them were manual workers. But Coleg Harlech has always endeavoured to bring its Welsh students into contact with those of other nations, and this year most of its terminal students and one or two of its sessional students came from such countries as Austria, Hungary, Germany, Denmark, Jamaica and the United States.

Whereas the intensity in industrial South Wales is only slightly more than the average for England, class units in the predominantly rural areas of Aberystwyth and Bangor are three times as numerous as they are in England in relation to population. This is as true of tutorial and sessional classes as it is of other kinds of work.

The subjects of study do not differ fundamentally from those conducted by the other extra-mural departments of the United Kingdom, except that Welsh history predominates over other kinds of history, and Welsh literature over other literatures. Although conditions have changed in recent years, the persistence of old interests is revealed by such facts as that the physical and biological sciences are completely overwhelmed by the humanities, that courses on religious subjects figure about twice as prominently as they do in England, and that there are relatively few courses in the visual arts.

One of the major problems is that of finding sufficient part-time tutors who are really qualified for university work, and this is particularly acute in the rural areas. Each College has five full-time tutors, and these, together with members of the intra-mural staffs, take a third of the classes. In the Aberystwyth and Bangor areas it is often difficult to take full advantage of the teaching staffs of the colleges because most classes within a radius of forty miles from the college centre are conducted in Welsh, a language which three-quarters of the intra-mural staffs do not understand. Each college is obliged to depend upon part-time tutors—lecturers in training colleges and theological colleges, school teachers and ministers of religion—for the bulk of the teaching. The need for a substantial expansion in full-time staffs remains.

INFLUENCE OF CERTAIN INORGANIC IONS ON THE HATCHING OF THE POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

By Dr. C. ELLENBY and A. B. GILBERT

Department of Zoology, King's College (University of Durham), Newcastle upon Tyne

PREVIOUS studies on the influence of salts on the hatching and emergence of larvæ of plant parasitic nematodes have approached the problem largely from the point of view of their possible osmotic effect[1,2]. However, the demonstration that the hatching-factor of the potato root eelworm possesses cardiotonic properties[3] suggested that the nature of the ions might be of importance. As already briefly announced[4], this we have found to be the case.

After a preliminary soaking in distilled water, cysts have been stimulated with solutions containing chlorides of sodium, potassium, calcium and magnesium, singly or in various combinations; each solution also contained a quantity of a concentrated hatching-factor preparation free from inorganic material, at a strength 1/5 of its optimum to give scope for increased or decreased hatching. A single-cyst technique was used[5] and a logarithmic transformation for statistical analyses[6]. In general, we have found that chlorides of the monovalent ions had a depressing effect, that is, hatching was lower than in the hatching factor alone; with the chlorides of the divalent ions hatching was higher. There was also some evidence of antagonism. No emergence took place in the salt solutions without hatching factor.

In most of our experiments, all salt solutions have been equivalent osmotically to 2 m*M* potassium chloride, so that it has always been possible to compare the different metallic ions independently of any osmotic effect. But in some experiments, a series of different concentrations has been employed, always so that it was possible to compare iso-osmotic solutions of the different salts. In some cases, we have controlled the possible influence of osmotic factors on the performance of the hatching factor alone by the addition of an appropriate quantity of glycerol.

Particular attention has been paid to potassium and calcium ions, in view of their known effect on heart action. The results of a series of experiments are summarized in Fig. 1. Although carried out at different times during 1957, the experiments were sufficiently similar to justify combining their results for purposes of presentation, and, in the diagram, each point is a mean value based on a number of experiments. Most of the experiments were carried out at concentrations equivalent to 2 and to 0·2 m*M* potassium chloride, the values for higher and lower concentrations being based on fewer experiments. The line representing hatching factor alone is based on nine lots of cysts in five separate experiments, four with hatching factor alone, and five with glycerol added to bring them to various concentrations

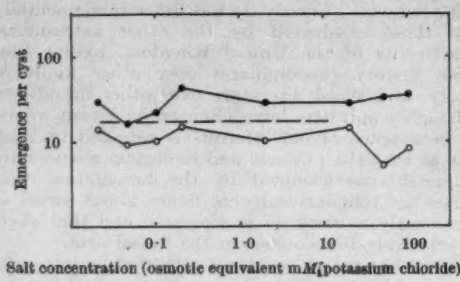

Fig. 1. Influence of potassium chloride and calcium chloride on hatching-factor activity. Values, being based on a logarithmic transformation, are essentially geometric means on a double log grid.

●—●, Hatching-factor + calcium chloride; ——, hatching-factor alone; ○—○, hatching-factor + potassium chloride

covering the range of the diagram up to 20 m*M* potassium chloride: there were no significant differences among these results, and the dotted line is based on an over-all mean.

Salt concentration had little effect, emergence being fairly uniform over the 5,000-fold range of concentrations examined; but, clearly, emergence was higher in solutions of hatching factor containing calcium ions than in those containing potassium ions. Of the sixteen lots of cysts tested with potassium chloride and hatching factor in the various experiments, fifteen gave hatches lower than their appropriate control; for calcium chloride, thirteen out of sixteen gave higher values. In fact, potassium usually about halved hatching and calcium doubled it. Since, in general, larval emergence is proportional to log hatching factor concentration[7], the over-all increase in emergence between potassium-containing and calcium-containing hatching solutions is equivalent to increasing the concentration of hatching factor about twenty times.

There is chemical evidence that, like the cardiac glycosides, the hatching factor contains an unsaturated lactone group[8]; for this reason, and because of its cardiotonic activity, it was suggested that it might act by influencing ion transport[8]. The influence of ions on hatching-factor activity is consistent with this view: it derives additional support from the results of experiments with quinidine sulphate. Whether quinidine-like agents act on cardiac muscle by reducing permeability to potassium ions[9], or by acting directly on the carrier mechanism[10], it is agreed that they influence ion transport in some way. It is therefore particularly interesting that quinidine sulphate has a powerful effect on the activity of the hatching factor in the presence of potassium ions. The addition of 3×10^{-6} *M* quinidine sulphate to a hatching-factor solution had no significant effect on its activity, emergence being 44 larvæ/cyst in hatching factor alone and 39·5 with the drug added. In hatching factor + 2 m*M* potassium chloride, hatching was reduced significantly to 17·2; however, the addition of 3×10^{-6} *M* quinidine sulphate to this solution completely nullified the potassium effect, emergence being 43·7.

These experiments were all carried out with cysts from the same source and, in repeat experiments, reasonably consistent results were obtained. However, lack of the same consistency with cysts of different origin suggested that the ionic contribution of the cyst contents might be of importance; and a number of experiments have been carried out which suggest that this may indeed be the case. In the first, four lots of cysts were soaked in solutions of sodium chloride, potassium chloride, calcium chloride and magnesium chloride, respectively, all iso-osmotic with 2 m*M* potassium chloride; a further lot was soaked in distilled water. After a fortnight, they were washed, and then all stimulated with hatching-factor solution. At the end of a week, emergence from the cysts soaked in the salt solutions was about three times as high as emergence from the cysts soaked in water. A repeat experiment, using iso-osmotic glycerol instead of water for the control, gave similar results. The effect decreased with time, so that, in some cases, the final hatches did not differ significantly. However, most of our experiments were carried out with sub-optimal concentrations of hatching factor and there is evidence that, with stronger solutions, the initial response is so much higher that a larger total hatch results. The effect can also be reduced by soaking the cysts in water for two weeks, after the salt soaking; but in some experiments it persisted after this and even after more rigorous treatment. In one experiment, cysts were removed from the salt solutions after a fortnight, washed, and allowed to dry at room temperature. They were maintained air-dry at 23° C. for a fortnight, soaked for a further two weeks in distilled water, and then stimulated with hatching factor in the usual way. Emergence from the originally salt-soaked cysts was significantly higher than from the originally water- or glycerol-soaked cysts otherwise treated identically.

Experiments on the ionic pretreatment of cysts are not sufficiently advanced for us to speak with confidence of differences due to particular ions. But there is evidence that such differences may exist. In the experiment just described, although the hatches from all the salt-soaked cysts were higher than controls, the differences were highest in the case of potassium chloride and sodium chloride, and the effects were apparently more persistent. Experiments are still proceeding, and a full account will be presented in due course, but already it can be said that it is not improbable that some of the differences in the behaviour of cysts from different sources may be due to the ionic environment to which they have been subjected. Possibly this factor may be partly responsible for the differences between cysts from different sources to which Fenwick has directed attention[7].

The experiments on the influence of ions were only made possible by the gift of a sample of a hatching-factor concentrate free from inorganic material; for this we offer our grateful thanks to Dr. G. J. Janzen, of the Proefstation voor Aardappelverwerking, Groningen. The work forms part of a programme supported by a grant from the Nuffield Foundation, for which we should also like to express our gratitude.

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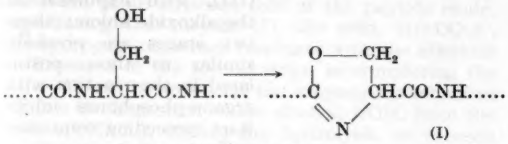
MECHANISM OF ESTERASE ACTION

Nature of the Reactive Serine Residue in Enzymes inhibited by Organo-Phosphorus Compounds

THERE is now overwhelming evidence[1] that the inhibition of cholinesterase, chymotrypsin and other esterases by di-*iso*propyl phosphorofluoridate and other organo-phosphorus compounds involves the phosphorylation of only one of the many serine residues present in the enzyme molecule. Serine itself does not react with di-*iso*propyl phosphofluoridate in aqueous solution[2], and there must be some special explanation for the exceptional reactivity of one of the serine residues in the sensitive enzymes. There are two obvious ways in which the reactivity of a serine side-chain may be enhanced: it may be influenced by some other side-chain (for example, histidine[1]) in its proximity, or it may be chemically modified.

One chemical modification of a serine side-chain to give a possibly more reactive grouping is cyclization to a Δ^2-oxazoline, (I):



(I)

The presence of Δ^2-oxazoline residues in intact proteins has been suggested before[3] and they have been postulated as intermediates in the N → O acyl migrations which occur when serine-containing peptides and proteins are treated with acid[4].

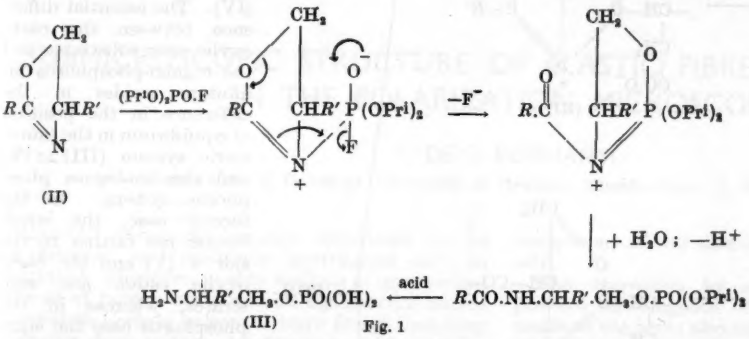


Fig. 1

We have, accordingly, studied the action of di-*iso*propyl phosphofluoridate in aqueous sodium bicarbonate at 37° on three representative Δ^2-oxazolines, namely, 2-methyl-Δ^2-oxazoline (II; R = Me; R' = H), 2-phenyl-Δ^2-oxazoline (II; R = Ph; R' = H), and 4-carbamoyl-2-phenyl-Δ^2-oxazoline (II; R = Ph; R' = —CO.NH$_2$). Although the last-named compound did not react, the two others did, the methyl compound more readily than the phenyl, giving products which yielded O-phosphoryl-ethanolamine (III; R' = H) on acid hydrolysis; control experiments showed that the reactions did not involve prior ring-opening to N-acyl-ethanolamines, followed by O-phosphorylation.

A reasonable course for the reaction is shown in Fig. 1. Our main argument, however, is not dependent on the validity of this mechanism.

As might be expected from the nucleophilic reaction of the oxazoline nitrogen postulated in the first stage, measurement of the basic strengths of the three Δ^2-oxazolines studied showed them to run parallel with the reactivity towards di-*iso*propyl phosphofluoridate, thus:

Δ^2-Oxazoline	Reactivity towards di-*iso*propyl phosphofluoridate	pK_a
2-Methyl-, (II; R = Me; R' = H)	++	5·5
2-Phenyl-, (II; R = Ph; R' = H)	+	4·4
4-Carbamoyl-2-phenyl-, (II: R = Ph; R' = CONH$_2$)	0	2·9

The decreases in basic strength observed on replacing a 2-methyl- by a 2-phenyl-group and on introducing an amide grouping at position 4 are expected consequences of the electron-attracting properties of the phenyl and amide groups.

It appears, from these results, that a Δ^2-oxazoline derivative will react with di-*iso*propyl phosphofluoridate in aqueous sodium bicarbonate, under conditions under which serine itself will not react, to give an O-phosphoryl-ethanolamine derivative, providing that it is sufficiently basic (pK_a not less than about 4). Whether or not a Δ^2-oxazoline peptide, as (I), will react under these conditions will depend on its basic strength; it seems likely that such a grouping will have a pK_a of just about 4, owing to the base-weakening effect of the adjacent peptide grouping, and may, therefore, be expected to react with di-*iso*propyl phosphofluoridate (and, presumably, also with analogous organo-phosphorus compounds) under such 'physiological' conditions.

We suggest, therefore, as a working hypothesis, that the singular serine residue present in the active centres of cholinesterase, chymotrypsin and other enzymes sensitive to di-*iso*propyl phosphofluoridate is, in fact, a Δ^2-oxazoline residue, as (I), and that it owes its unique reactivity to this chemical modification. Possible implications of this hypothesis for the mode of action of esterases are elaborated in the following communication.

Details of this work will be published elsewhere in due course; in the meantime, the experimental consequences are being actively pursued by one of us (H. N. R.) in collaboration with others.

G. R. Porter
H. N. Rydon
J. A. Schofield

Manchester College of Science and Technology,
Manchester, 1.

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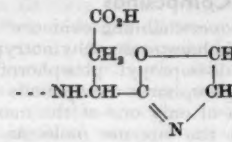
A Possible Mechanism of Action of Esterases inhibitable by Organo-Phosphorus Compounds

It has been shown that, in no less than six esterases (trypsin[1,2,5,6], chymotrypsin[1,2,5,7], red-cell cholinesterase[4], serum pseudo-cholinesterase[4], red-cell ali-esterase[4] and liver ali-esterase[4]), the uniquely reactive serine residue (-Ser*-), which is phosphorylated when the enzymes are inhibited by organo-phosphorus compounds, is situated in the sequence -Gly-Asp-Ser*-Gly-; it seems reasonable to suppose that this particular sequence must play an important part in the esteratic action of these enzymes. In this sequence, only the aspartic acid and serine side-chains are chemically reactive, and it thus seems likely that the key to the esteratic activity lies in the sequence -Asp-Ser*-. In the preceding communication, it has been suggested that the reactive 'serine' is, in reality, a Δ^3-oxazoline; if this is accepted, the structure of the key sequence is:



and it is the purpose of the present communication to show how this structure might operate as an esteratic centre.

The suggested esteratic process is shown in Fig. 1. The first stage involves nucleophilic attack of the oxazoline nitrogen on the ester to form the intermediate (II), which then is rearranged to the cation (III), with expulsion of the alkoxide anion; these two stages are precisely similar to those postulated in the reaction with organo-phosphorus inhibitors (preceding communication).

The cation (III) is in tautomeric equilibrium with its isomer (V), by way of the geometrically feasible transition state (IV). The essential difference between the carboxylic-ester substrates and the organo-phosphorus inhibitors resides in the difference in the position of equilibrium in the tautomeric system (III) $\rightleftarrows$ (V) and the analogous phosphorus system. In the former case, the equilibrium lies farther to the side of (V) and the carboxylic esters are substrates, whereas in the phosphorus case the equilibrium is more in favour of the analogue of (III), in consequence of which the organo-phosphorus compounds are inhibitory, undergoing the further stages of the esteratic reaction sequence only very slowly[8]. The postulated difference in the point of equilibrium in the two cases is supported by the observation that treatment of Δ^3-oxazolines with di-*iso*propyl phosphorofluoridates gives O-phosphoryl-ethanolamine derivatives (preceding communication), whereas

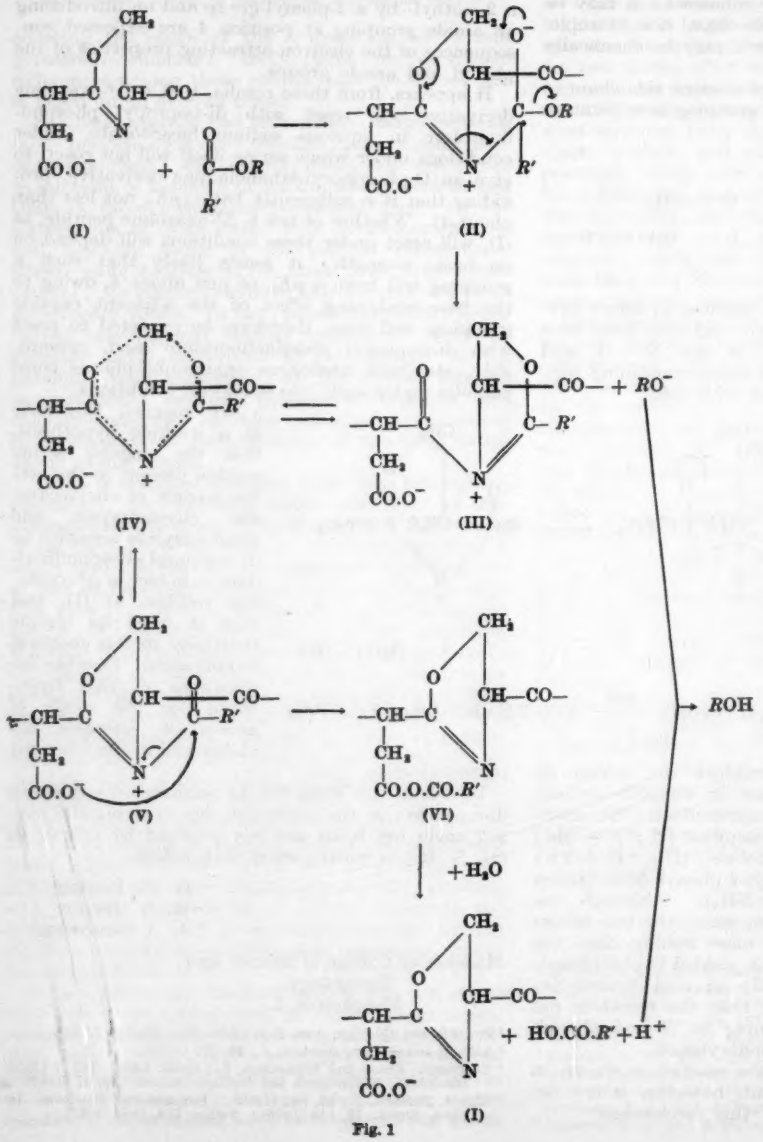


Fig. 1

carboxylic acid halides yield ethanolamine derivatives with the new acyl group attached to nitrogen[9].

In the next stage, the ionized aspartic acid side-chain, drawn by electrostatic forces to the neighbourhood of the positively charged nitrogen and so favourably placed to do so, attacks the carbonyl group of the $\rangle\overset{+}{\mathrm{N}}.\mathrm{CO}.R'$ grouping. This process (compare the acylating properties of, *inter alia*, N-acyl-pyridinium salts[10], N-acetyl-imidazole[11] and N-phospho-imidazole[12]), in which the charges are neutralized, leads to the anhydride (VI), in which the acyl residue is now attached, temporarily, to the aspartic acid side-chain. It is noteworthy that, in strongly acid solution, when the aspartic acid side-chain will not be ionized, it is possible to isolate an acetyl-chymotrypsin from the products of the action of chymotrypsin on *p*-nitrophenyl acetate[13]; this acetyl-chymotrypsin behaves as an acetylating agent, as the protonated form of V (R' = Me) might well be expected to do.

Finally, the anhydride (VI) undergoes hydrolysis, possibly assisted by a suitably placed histidine side-chain in an adjacent fold of the peptide chain, regenerating the enzyme (I), the acid, HO.CO.R', and hydrogen ion, which combines with the alkoxide ion liberated in the second stage, so completing the cycle. It will be seen that the suggested mechanism requires the liberation of the alcohol, *R*OH, from the ester, R'.CO.O*R*, undergoing hydrolysis, to precede the liberation of the acid, $R'.\mathrm{CO_2H}$; this is in agreement with the experimental finding[14] that, in the hydrolysis of *p*-nitrophenyl acetate by chymotrypsin, a relatively rapid liberation of *p*-nitrophenol is followed by a much slower liberation of acetic acid.

The mechanism of esterase action advanced above is in agreement with many previous experimental findings and has the advantage of affording an interpretation of the existence of a common amino-acid sequence around the reactive 'serine' in many esterases, which has already been commented on, but not interpreted, by others[4,15]. Whether or not it eventually proves to be valid, it does at least suggest further experimental studies, some of the more obvious of which will be undertaken in these laboratories.

H. N. Rydon

Washington Singer Laboratories,
University of Exeter.

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SUBMICROSCOPIC STRUCTURE OF ELASTIC FIBRES AS OBSERVED IN THE POLARIZATION MICROSCOPE

By Dr. G. ROMHÁNYI

Department of Pathology, University of Medicine, Dischka Gy.-u. 5, Pécs, Hungary

ELASTIC fibres are usually considered to be isotropic, and positively birefringent only on stretching[1]. However, in thin sections of unstretched material of human aorta and ox ligamentum nuchæ mounted in Canada balsam, I have found that they invariably show positive birefringence with retardations of about 8 mμ and 18 mμ, respectively. In a previous communication[2] I have shown that this positive birefringence of the elastic fibres in the human aorta changes into negative birefringence with retardations of about 16 mμ, when the sections are mounted in aniline after fixation in formalin, alcohol, Bouin, or Carnoy, dehydration of the deparaffinized sections in absolute alcohol, and clearing in terpineol or xylol (for further details see ref. 2).

The elastic fibres are slightly negatively birefringent already in a mixture of aniline (20 per cent) and terpineol, showing a refractive index, *n*, of 1·500 in an Abbé refractometer. Thus the negative birefringence of the elastic fibres in aniline cannot be regarded as being due to the relatively high refractive index of aniline ($n = 1{\cdot}58$), but rather as the optical evidence of its orientated adsorption to a structural component of the elastic fibres arranged longitudinally.

The depression by aniline to isotropy of the positive birefringence of the collagen fibres in the media of the aorta also suggests an orientated binding of aniline to the collagen fibres. Furthermore, in media with relatively high refractive indices obtained by mixing bromphenol ($n = 1{\cdot}57$) and monobromnaphthalene ($n = 1{\cdot}66$) there is no change of the positive birefringence of the elastic fibres to negativity.

The elastic fibres of the aorta of children show an axial isotropic band in aniline (Fig. 1) which, like that seen in some plant fibres[3], could be regarded as optical evidence of a submicroscopic spiral texture resulting from the compensation of the effects of the coiled filaments at their crossings at right angles in the fibre axis. Also, in aniline the elastic fibres of the ligamentum nuchæ show a broad axial compensation band as optical evidence of their spiral submicroscopic structure. These findings are in agreement with the earlier electron microscopic observation of a spiral texture of the elastic fibres

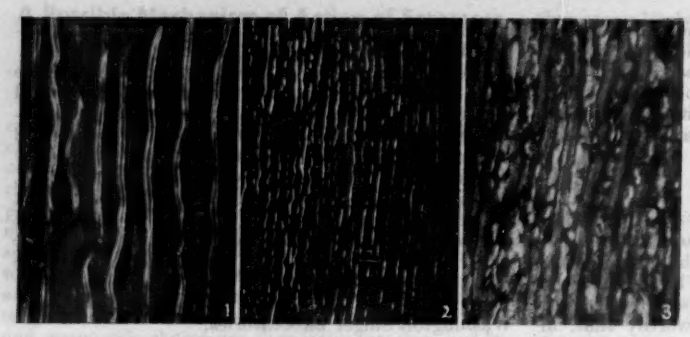

Fig. 1. Paraffin section of the aorta of a newborn infant mounted in anilin. The elastic fibres show intensive negative birefringence with an axial isotropic band

Fig. 2. Aorta of an adult. After treatment with testicular hyaluronidase for 2 hr. and staining with toluidin blue-ferricyanide at pH 4·0. The elastic fibres show intensive negative birefringence

Fig. 3. Aorta of an infant. Phenol reaction. The elastic fibres show slight negative birefringence with a poorly visible darker axial compensation band. They are surrounded by collagen fibres of stronger negative birefringence

in the ligamentum nuchæ and the skin[4]. With age the axial compensation band becomes thinner or disappears, which is probably due to an elongation of their inner spiral texture.

In recent studies with a new anisotropic staining method (buffered toluidine blue solutions at *p*H 7·0–2·6, followed by treatment with 5 per cent ferricyanide solution) I have found that, as a sign of their submicroscopic fibrillary structure, not only the mucoid components of the connective tissue ground substance[5] but also the elastic fibres of the human aorta show negative birefringence in green, yellow and red light, while the latter also displays isotropy in blue light, and anomalous colours ranging from dark blue to greenish-yellow, depending on the amount and sign of compensation. Thus, the elastic fibres stained with toluidine blue exhibit an optical behaviour similar to that of the collagen fibres similarly stained, which has recently been shown by Brewer[6] to be the result of an orientated adsorption of the dye molecules on the collagen fibres with their light-absorbing bonds at right angles to the fibre axis. The collagen fibres gave their staining reaction with toluidine blue above *p*H 4·1. However, I found that, like the acid mucopolysaccharides of the aortic media, the elastic fibres showed anisotropic staining with toluidine blue ferricyanide in regions more acidic than those of the collagen fibres. Thus the anisotropy of the toluidine blue-stained elastic fibres might be interpreted as the result of an orientated binding of the dye molecules on the structural pattern of the mucoid components in the elastic fibres.

It is known that the elastic fibres in the aorta of the newborn are faintly basophilic[7]. That is why, when stained with toluidine blue, they show only weak anisotropy. But their anisotropic staining is markedly enhanced after pretreatment with potassium permanganate (as used in the silver impregnation techniques), which renders the elastic fibres and other tissue elements more basophilic by increasing the number of negatively charged side-groups on the substrates. This also applies to the faintly basophilic elastic fibres in the skin and in ox ligamentum nuchæ. It is interesting that, after treatment with permanganate, the enzymatic susceptibility to elastase (kindly supplied by Prof. I. Banga of Budapest) of the elastic fibres of human aorta and ox ligamentum nuchæ, measured by the time of their dissolution, was more than 100 times that of the controls.

In view of the structural complexity of the elastic fibres, evidence was sought by polarization microscopy bearing on the question of the possible collagen-like nature of the filaments demonstrated in elastin by electron microscopy[8]. In this connexion it is significant that the elastic fibres invariably change their positive birefringence to negative birefringence in sections mounted in phenol. In view of the high specificity of Ebner's phenol reaction for collagen[9]—given not only by mesenchymal but also by ectodermal (cuticular, lens capsule) collagens and by the collagen fibrils reprecipitated *in vitro*—it seems reasonable to assume that the protein filaments embedded in the matrix substance of the elastic fibres are in close structural relation to collagen-type proteins.

Polarization microscopy thus provides the possibility of distinguishing in the elastic fibres two structural components: a protein demonstrable by the phenol reaction, and a mucopolysaccharide demonstrable by the toluidine blue ferricyanide method, both being present in a longitudinal or spiral arrangement. However, these two methods cannot be applied simultaneously to the same preparation. Toluidine blue-stained fibres when mounted in phenol lose their stain and show only the effect of the phenol reaction. It seems probable that close structural interrelationships between the two components and steric hindrances inhibit the simultaneous binding of the two components on the same structural basis.

The structural pattern of the elastic fibres suggested on the basis of these polarization microscopic findings is in good agreement with Hall's[10] model, which is based on electron microscopic and chemical observations of the enzymatic dissolution process of elastin. However, polarization microscopy has shown that, apart from their inner spiral architecture, a considerable portion of the mucoid component of the elastin fibres is not amorphous but longitudinally orientated along the fibres. This may possibly result from an interaction of the mucoid component with the protein filaments arranged longitudinally.

A more detailed account of these observations will be published elsewhere.

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LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Ferromagnetism of Meteorites

THE aim of this communication is to describe some preliminary results on the magnetic behaviour of some stony meteorites, namely, Allais, Cold Bokkeveld and Orgueil. Paramagnetic resonance absorption has been used in order to be able to investigate ferromagnetic resonance and eventually paramagnetic particles or free radicals at the same time. These were not unexpected on account of the relatively high content of organic material of the selected meteorites[1]. The apparatus used was a Varian *X*-band microwave spectrometer. In no case was a sharp electron spin resonance observed, which seems to indicate that paramagnetic species are not present in significant concentrations. Instead a broad line occurred, which is presumed to arise from ferromagnetic resonance. The location of the maxima and the line-widths between the inflexion points are shown in Table 1.

Table 1

Name	Max. H_0 (œrsteds)	Line widths (œrsteds)
Allais	3,100	2,320
Cold Bokkeveld	3,100	2,460
Orgueil	3,100	2,250

The spectroscopic splitting factor (g), deduced assuming a random distribution for the crystallites, amounts to 2·19. This is not in disagreement with the corresponding values observed for nickel magnetite[2] and ferrites[3]. If the results of the elemental chemical analyses[4] are interpreted in terms of mineral compounds, it may be inferred that these substances are present.

It is to be noted that we have now at our disposal an objective method for studying the evolution of ferromagnetic content through the group of stony meteorites. Further research on this problem is now in progress.

We are greatly indebted to Father J. Junkes, director of the Spectroscopic Laboratory of the Vatican Observatory, for the loan of specimens of meteorites. Our thanks are also due to Varian Associates for putting a spectrometer at our disposal.

JULES DUCHESNE
JOSEPH DEPIREUX

Institut d'Astrophysique,
University of Liège,
Belgium.
Aug. 1.

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Removal of Gases in High Vacuum Systems by Metal Abrasion

CHEMICAL reaction with metal films (gettering) is a well-known process for the removal of free gas in vacuum devices. However, removal can cease once a thin layer of reaction product has formed. Thus the layer of oxide which forms on fresh aluminium surfaces at room temperature is protective[1], and so are the layers of oxide that form on the initially reactive metal, titanium[2]. Similar protective layers of compounds other than oxides also occur—see, for example, a recent review[3].

In order to overcome such limitation upon capacity for taking up gas, pumps have been designed[4] in which titanium is evaporated continuously, to provide films having fresh surfaces highly reactive with gas molecules. However, to evaporate titanium fast enough for the pump to take up gas at a useful rate (say, a few litres/μsec.), the metal must be raised above its melting point (1,670° C.), as vapour pressure data[5–7] show. Unfortunately, at such high temperatures, titanium is so reactive that there are few suitable materials from which to make hearths for holding the molten metal.

As alternative to overlaying the protective reaction products with freshly evaporated titanium, a promising idea is abrasion of the oxide, or similar layer, to expose the fresh titanium beneath. Only a very thin layer of oxide has to be removed, so scratching rather than, say, turning seems appropriate. Some preliminary experiments demonstrating the reaction of gas with a freshly abraded surface of titanium will now be described.

A trough was made from sheet titanium and in it were put a few lumps of some suitable abrasive, such as 'Carborundum' (Carborundum Co., Ltd., Manchester 17), or some pieces of an old metal file. The titanium trough was mounted in a glass bulb to which was attached both an ionization gauge for measuring the pressure of free gas and a porous ceramic appendage through which fresh free gas could be introduced at will from a surrounding jacket that could either be filled with known gases or evacuated. As a start the whole device was evacuated, baked to remove water vapour, and sealed off containing free gas at some pressure less than 10^{-6} mm. mercury. Then gas was admitted through the porous ceramic tube just fast enough to maintain the pressure at, say, 10^{-4} mm. mercury, in spite of the reaction of the gas with the hot tungsten filament and some loss to the walls (clean-up). (With oxygen the loss by reaction with the filament is appreciable—see, for example, ref. 8—but with nitrogen is negligible). When the lumps of abrasive were shaken about in the trough of titanium, or the bits of file dragged back and forth therein by means of an external magnet, the pressure fell rapidly, in less than a minute, to 10^{-6} mm. mercury. By admitting more fresh gas this fall could be demonstrated repeatedly in any particular tube. After many runs using lumps of abrasive so much powdered debris covered the bottom of the trough that gas was not taken up as readily as at the start. But in such a tube the previous best performance was maintained by dragging about the bits of file with a magnet; for in this way loading upon, and mechanical damage to, the titanium was greater.

Since air contains about 1 per cent of argon, it might have been expected that it would be impossible to reduce the pressure again below about 10^{-6} mm. mercury, and that after each successive admission of fresh gas the background pressure of free argon would increase. But this did not happen. It is true that the most rapid fall of pressure upon shaking and scratching occurred for the first hundredfold reduction; but it continued thereafter more slowly,

while the ionization gauge was run. Such slow cleaning-up of the last 1 per cent remnant of air occurs probably through an exchange of argon with other gases hidden in the walls[9] and electrodes[10] or by absorption as in getters protected by argon atmospheres[11,12]. A more rapid removal of argon would be essential for improving the performance of any abrasion pump required to remove air entering continuously through leaks, as in demountable equipment; but the present form is adequate for removing gas coming from virtual leaks in sealed-off tubes like those used here.

M. E. Haine
E. W. R. Francis
R. N. Bloomer

Research Laboratory,
Siemens Edison Swan, Ltd.,
West Road,
Templefields, Harlow.
Sept. 4.

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Anomalous Dissociation of Molecular Hydrogen by Electron Impact

An electron colliding with a hydrogen molecule can raise it to an excited state from which it may return to the ground-state by dissociating into atoms with or without emission of a light quantum. Numerous experiments with electrons up to about 30 V. energy have been carried out, but their interpretation was of heuristic value only. Neither the critical potentials for the various processes nor the dependence of the probability of a process on the electron energy was known with sufficient accuracy, and even the relative contribution of the various processes to dissociation was a matter of conjecture.

The problem was therefore attacked anew, both experimentally and theoretically. Recent observations by Corrigan and von Engel[1] show that the main contribution to dissociation comes from raising the molecule to the repulsive lowest triplet state, from which it dissociates into two hydrogen atoms in the ground-state, each with kinetic energy of a few volts; that is, no light emission occurs. This transition sets in theoretically at about 8·8 V., though earlier observations suggested an onset potential of 11–11·5 V.

Present theoretical work provides a possible explanation of certain anomalies in the region of the onset potential. In addition, there is some measure of quantitative agreement between the theoretical results of the probability of the above transition and new measurements with electron swarms.

A variational method was used to calculate the inelastic scattering cross-section associated with the transition $^1\Sigma_g^+ \rightarrow {}^3\Sigma_u^+$, and 120 values of the cross-section have been obtained, that is, 20 values with a final energy of the electron from 0 to 18-V. at intervals of 0·8 V. and six values with an energy loss of 8·6–15·5 V. at 1·4-V. intervals.

To obtain the dissociation cross-section as a function of the initial energy of the electron, these values should be averaged over the vibrational motion. However, if it is assumed that the result of this would be to select effectively the curve corresponding to energy loss of about 10–10·5 V., that is, one at the centre of the Franck–Condon range, then useful preliminary results can be found without detailed averaging.

All the six curves obtained have three features in common: first, in the range 0–0·8 V. above threshold, the cross-section is very small; it then rises in the range 0·8–1·7 V. above threshold by a factor of 200 or more and yet not giving values greater than the theoretical maximum which follows from the conservation of charge. Secondly, from 1·7 to 5 V. above threshold, the cross-section drops to a minimum value; and then, thirdly, in the range 5–18 V. above threshold it rises to a second maximum and falls off again.

The following table gives the values of the cross-section for an energy loss of 10 V., the critical potential for the chosen fixed interatomic distance between the nuclei ($\pi a_0^2 = 8{\cdot}8 \times 10^{-17}$ cm.^{2}):

Initial electron energy (V.)	10	10·8	11·7	12·5	15·8	16·7	21·7	22·5	26·7
Cross-section (πa_0^2)	0	$2{\cdot}4\times10^{-3}$	0·7	0·52	0·19	0·19	0·33	0·33	0·22

If the cross-section curve for 10 V. energy-loss is representative, then the theory predicts that the cross-section is very small up to 10·8 V. and rises by a large factor of order 100 to a maximum at about $11{\cdot}9 \pm \frac{1}{4}$ V. (since the probable energy-loss is between 10 and 10·5 V.). Because of the extremely small cross-section for the first 2 V. above the true onset at 8·8 V., we find what might be called an 'effective onset potential' between 10·8 and 11·3 V. which would be observed by experimenters using an apparatus of limited sensitivity. This result is in agreement with the earlier observations cited above. Whereas the anomalous onset has been speculatively explained[2] as entirely due to the vibrational averaging, it is now thought that the anomaly can be resolved into an electronic and a vibrational contribution both of the same order of magnitude.

Recently, Corrigan and von Engel[1] have measured dissociation coefficients in hydrogen, that is, the number of dissociations per centimetre of path in the field direction using an electron swarm in a uniform electric field of magnitude less than or equal to 100 V./cm. at 1 mm. mercury pressure. If the energy distribution of the electrons in the swarm is assumed to be Maxwellian, then the above theoretical results are in accord with the rate of dissociation observed.

I wish to acknowledge the guidance of Prof. H. S. W. Massey in my calculations.

L. A. Edelstein

Clarendon Laboratory,
Oxford.
July 30.

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Anthocyanins of Blood Oranges

THE fleshy endocarp of the blood orange, a variety of sweet orange, *Citrus sinensis*, contains a water-soluble red pigment which Matlack[1] showed to be anthocyanin in nature.

Factors influencing the production of anthocyanin in Sicilian varieties have been discussed by Carrante[2]; estimates of the amounts found in the juice have been made by Ajon[3]; and Patanè has published a method for its partial isolation[4]. However, no formal identification has apparently yet been made. Evidence is presented here to show that cyanidin-3-glucoside is the major pigment of the Moro variety of the blood orange, with a second pigment, probably delphinidin-3-glucoside, as a minor component.

Extraction of the pulp (2 kgm.) with methanol containing 1 per cent hydrochloric acid was followed by precipitation of the pigment as the lead complex. The precipitate was decomposed with hydrochloric acid, and the recovered pigments were taken up in butanol to which 4 volumes of benzene were then added. The solution was extracted with one volume of 1 per cent hydrochloric acid. The difficultly soluble hesperidin remained in the organic layer from which it was slowly deposited on standing. Purification was effected by cellulose column chromatography with the organic phase of butanol/2*N* hydrochloric acid as solvent, used for separation of the black currant anthocyanins[5]. Three bands appeared on the column as elution proceeded: a rapidly moving brown band, a scarlet band, and a small slowly moving mauve band. The eluate was collected in 50 ml. fractions and a graph of the optical density at 535 mμ against fraction number indicated that the third pigment represented only about 1 per cent of the total.

The pigment in the first band was concentrated by precipitation as the lead complex and the other two by absorption on a second cellulose column as already described[5]. Paper chromatography of these concentrates and their hydrolysis products gave the following results, confirmed where possible by absorption spectra and colour tests.

(*a*) The pigment in band 1 consisted largely of red-brown material running at all times with the solvent front. Also present were traces of an anthocyanidin, chromatographically identical with cyanidin, obtained by hydrolysis of the second strawberry pigment, cyanidin-3-glucoside[6]: R_F in butanol/2*N* hydrochloric acid, 0·75; in Forrestal solvent, 0·49.

(*b*) The pigment in band 2, amounting to about 95 per cent of the total, was a cyanidin derivative, the aglycone obtained on hydrolysis by treatment with 5 per cent perchloric acid for 30 min. at 50° C. being chromatographically identical with cyanidin. The anthocyanin behaved as a 3-glycoside, co-chromatographing with the second strawberry pigment in four solvent systems: R_F in butanol/2*N* hydrochloric acid, 0·26; in butanol/acetic acid/water, 0·38; in *m*-cresol/acetic acid/water, 0·34; in Forrestal solvent, 0·74.

(*c*) The pigment in band 3 was a delphinidin derivative, and chromatographed as a 3-glycoside in the four solvent systems given above, with R_F values of 0·16, 0·21, 0·16 and 0·65, respectively. The aglycone obtained on acid hydrolysis was chromatographically identical with delphinidin, the hydrolysis product of delphin, obtained from blue delphiniums and purified by column chromatography: R_F in butanol/2*N* hydrochloric acid, 0·45; in Forrestal solvent, 0·28.

(*d*) Glucose was the only sugar present in the hydrolysates from the two glycosides, co-chromatography with glucose producing no separation in three solvent systems: R_F in *n*-collidine, 0·38; in ethyl acetate/water/pyridine (2 : 2 : 1), 0·30. R_x in water-saturated *n*-amyl alcohol, 0·42 (R_x of xylose, 1·00). Galactose in these solvent systems gave values of 0·35, 0·25 and 0·33, respectively.

From the hydrolysate of the main pigment an osazone was prepared, melting point 205° C.; no depression when mixed with authentic glucosazone, melting point 207° C.; but depressing the melting point of authentic galactosazone (197° C.) by 15–20 deg.

These results identify the main pigment as cyanidin-3-glucoside, confirmed by the determination of the aglycone : glucose ratio, using the method and the molecular extinction coefficient given by Nordstrom[7].

Identification of the second glycoside as delphinidin-3-glucoside is based on its behaviour on the paper chromatogram, partition between solvents, colour tests, and chromatographic behaviour of the sugar moiety.

These observations apply specifically to the Moro variety. The main pigment of three other varieties, Tarocco, Florida and Ruby Red, is indistinguishable chromatographically from that of the Moro, and no major varietal difference seems likely in anthocyanin pigmentation.

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B. V. CHANDLER*

Department of Food Technology,
University of California,
Berkeley, California.

Aug. 5.

* Permanent address: C.S.I.R.O., Division of Food Preservation, Homebush, N.S.W., Australia.

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Incorporation of Radioactive Sulphur by *Thiobacillus thioparus*

IN the course of our investigations on the metabolism of sulphur in the autotrophic bacteria *Thiobacillus thioparus* we have found that this micro-organism metabolizes only the outer sulphur atom of thiosulphate. The inner sulphur atom probably does not enter the bacterial cell and is left in the medium as a sulphate ion[1]. We carried this investigation further with thiosulphate labelled in the outer position with sulphur-35 ($Na_2O_3S^{35}S$), tracing the pathway of the radioactive sulphur in the bacterial cells.

The pool of free amino-acids and related compounds were investigated in the following manner: the ethanol extract from a culture of *Th. thioparus* which had been incubated for 24 hours with radioactive thiosulphate was separated by paper electrophoresis and chromatography, after which autoradiograms were made. We proved that radioactive sulphur is incorporated into cysteine, cystine and methionine as well as in cysteic and cysteinesulphinic acids. A few other radioactive spots were also

established. One of these sulphur compounds was identified as α-thiooctanoic acid, by its R_F value and from the results of microbiological tests with *Streptococcus faecalis*[2].

The whole cells of bacteria incubated in this way were hydrolysed, and the product was fractionated by paper electrophoresis and chromatography. All typical amino-acids were demonstrated in the hydrolysate; dicarboxylic acids, alanine, threonine, glycine, serine and leucine were present in particularly large amounts. We found the same sulphur amino-acids as in the ethanol extract. Besides these amino-acids some unidentified sulphur compounds were found.

Definitely different results were obtained during investigation of the alcohol extract and whole-cell hydrolysate, when the bacteria were incubated with radioactive thiosulphate for 1–10 min. only. The chromatographic analysis in this case, of course, gives the same results as for bacteria incubated with thiosulphate for 24 hours; the difference is in the autoradiograms in which no ninhydrin-positive substance has any measurable radioactivity. Instead of the known radioactive sulphur amino-acids, a radioactive spot appears corresponding to compounds which do not migrate electrophoretically at *p*H 2·7, and which do not stain with ninhydrin. The radioactivity of these substances increases with the duration of incubation. The unidentified substances which give rise to this spot are a mixture of at least two compounds which can be separated chromatographically in the system *n*-butanol—acetic acid—water (5 : 4 : 1), the R_F values of which are 0·23 and 0·52.

This and former experiments[1] lead us to suggest that the outer sulphur atom of thiosulphate reacts with some organic acceptor in the bacterial cells, forming a compound (not yet identified) detectable during the early stages of incubation with thiosulphate. This compound represents one of the steps in the metabolism of sulphur in the cell. The hypothetical organic acceptor probably reacts not only with the sulphydryl group of thiosulphate but also with the same group in other sulphur compounds. This idea arises from our experiments on *Th. thioparus* incubated in a medium containing cysteine labelled with sulphur-35. The culture of *Th. thioparus* does not develop when the sole source of oxidizable sulphur is cysteine; it only does so in the presence of thiosulphate. When *Th. thioparus* is incubated in a medium with unlabelled thiosulphate and labelled cysteine it is possible to demonstrate that after some time the alcohol extract and whole-cell hydrolysate contain the same radioactive sulphur compounds as were found after incubation with radioactive thiosulphate alone. In the medium, radioactive, elementary sulphur and radioactive sulphate ions also appear as oxidation products of cysteine sulphur. These radioactive substances cannot be solely the products of non-biological reactions between cysteine and thiosulphate, because such reactions would give rise to other compounds (following communication); nor do they originate from an isotope exchange reaction.

B. Skarzynski
W. Ostrowski

Department of Physiological Chemistry,
Medical Academy,
Cracow.
July 17.

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Reactions of Thiosulphate with Cysteine

During investigations of the metabolism of sulphur compounds in autotrophic bacteria and in higher animals, it was observed that thiosulphate reacts spontaneously with cysteine. This reaction has been studied fairly extensively, revealing some facts which may be of interest for understanding the behaviour of thiosulphate *in vitro* and *in vivo*.

The experiments reported below were performed in an apparatus consisting of a reaction vessel with a side-arm connected to an absorption vessel by a ground-glass joint. A stopcock in the joint connected the apparatus to a vacuum pump and the reactions were performed *in vacuo*.

Hydrogen sulphide was trapped by a suspension of cadmium hydroxide. Chromatograms were developed in acetic acid in phenol saturated with an aqueous solution containing 6·3 per cent sodium citrate and 3·7 per cent sodium dihydrogen phosphate. The spots were detected by ninhydrin staining. All reagents were of analytical grade.

(1) Thiosulphate (1 m.mole or more) was mixed with a solution of cysteine (1 m.mole in 5 ml.) suitably buffered with carbonate or phosphate to *p*H 7–7·4, and the mixture was left overnight at room temperature. The appearance of yellow cadmium sulphide in the absorption vessel indicated that hydrogen sulphide was evolved; at the same time a white substance was precipitated.

The hydrogen sulphide originates solely from the outer sulphur of thiosulphate, as can be shown by experiments with radioactive thiosulphate or cysteine. The hydrogen sulphide produced was radioactive only when thiosulphate labelled with sulphur-35 in the outer position ($Na_2O_3S^{35}S$) was used; it was completely inactive when the radioactivity was initially in the cysteine sulphur.

The white precipitate was identified as cysteine, but the amount represented only part of the cysteine originally present. This was interpreted as meaning that some of the cysteine was converted to a different compound. In order to study this problem the solution was investigated chromatographically. On the chromatograms there was only one well-marked ninhydrin-positive spot, with R_F value 0·04–0·05. (In the experimental conditions cysteine was deposited in too small a quantity to give a coloured spot). This spot was identical with that given by cysteinesulphonic acid prepared by Clarke's method[1].

The formation of hydrogen sulphide, cystine and cysteine–sulphonic acid as products of the reaction between thiosulphate and cysteine can be formulated as follows:

$$R - SH + {}^{-}S - SO_3^- \rightarrow R - S - SO_3^- + HS^-$$
$$R - S - SO_3^- + R - SH \rightleftarrows R - S - S - R + HSO_3^-$$

where R represents the aminopropionic radical $-CH_2.CHNH_2.COOH$.

Glutathione and dimercaptopropanol react also with thiosulphate with production of hydrogen sulphide, but the other products were not investigated.

(2) When the solution resulting from the reaction described above was warmed for several hours at 60° C., the precipitate of cystine dissolved, and the chromatograms of the solution exhibited, besides the cysteinesulphonic acid spot, another spot with R_F 0·08–0·1. This spot is supposed to be due to a new

compound, which may be called cysteinethiosulphonic acid: R—S—S—SO_3H. Its formation can be explained by the reaction:

$$R\text{—S—S—}R + {}^{-}\text{S—SO}_3^{-} \rightarrow R\text{—S—S—SO}_3^{-} + R\text{S}^{-}$$

This supposition is supported by the fact that a warmed solution of cysteine and thiosulphate tested chromatographically also gives two spots located in exactly the same positions as those described above. The second reason is that a compound with R_F value 0·08–0·1 is formed during the oxidation of a cysteine and thiosulphate mixture with iodine. In this case there are three possible parallel reactions:

$$2R\text{—SH} + I_2 \rightarrow R\text{—S—S—}R + 2H^+ + 2I^-$$
$$2\text{-S—SO}_3^- + I_2 \rightarrow {}^{-}\text{O}_3\text{S—S—S—SO}_3^- + 2I^-$$
$${}^{-}\text{S—SO}_3^- + R\text{—SH} + I_2 \rightarrow R\text{—S—S—SO}_3^- + H^+ + 2I^-$$

The third reaction produces cysteinethiosulphonic acid, which can be identified chromatographically.

(3) The reaction of cysteine with tetrathionate is connected with the facts described above. When solutions of these compounds (mM : mM) were mixed together a white precipitate was quickly formed, which was identified as cystine, but other products are also formed. Curiously enough, the chromatograms of the solution revealed the existence of three spots located near one another. The two lower spots corresponded to those of cysteinesulphonic acid and cysteinethiosulphonic acid, the third presumably belongs to a compound which may be called cysteinedithiosulphonic acid, R—S—S—S—SO_3H. The reaction which led to the formation of this acid may be formulated:

$$R\text{—SH} + {}^{-}\text{O}_3\text{S—S—S—SO}_3^- \rightarrow R\text{—S—S—S—SO}_3^- + HSO_3^-$$

If the formulations of the reactions described above are correct, a way is opened for the synthesis of a new class of compounds which may be called cysteinepolythiosulphonic acids. Further studies are in progress.

Tadeusz W. Szczepkowski

Department of Physiological Chemistry,
Medical Academy,
Cracow.

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Inhibition of Cholinesterase Hydrolysis of Dihydromurexine by Lysergic Acid Diethylamide and its 2-Bromo Derivative: a Selective Relationship

Previous investigators have shown that human plasma cholinesterase is strongly inhibited by both lysergic acid diethylamide (LSD) and its 2-bromo derivative (BOL). The cholinesterases of other species were less sensitive to the effects of both these substances[1–3]. Recent reports described certain newer choline esters, namely, imidazoleacrylylcholine[4,5] (murexine), imidazoleacetylcholine[6] and imidazolepropionylcholine (dihydromurexine) which are hydrolysed most readily by human plasma cholinesterase[7–10]. These observations have led us to evaluate the possible relationship between cholinesterase inhibition by lysergic acid diethylamide and BOL and cholinesterase hydrolysis of dihydromurexine. Dihydromurexine was chosen as the model substrate because it is the most active of the three esters.

Table 1. Hydrolysis of Butyrylcholine and Imidazolepropionylcholine by Sera of Several Species and the Inhibitory Effects of Lysergic Acid Diethylamide

1 ml. of 1 : 10 dilution of serum; 0·5 or 1 ml. of 0·1 M phosphate buffer pH 7·2; 0·5 ml. of inhibitor to give a final concentration of 8×10^{-4} M; 0·5 ml. of butyrylcholine (800 μgm./ml.) or 0·5 ml. of equivalent amount of dihydromurexine; temperature, 37° C.; incubation time, 10 min.; total volume, 2·5 ml. Choline esters were measured by Hestrin's method (ref. 11)

Substrate	Butyrylcholine		Dihydromurexine	
Inhibitor / Species	−LSD	+LSD	−LSD	+LSD
Cat	*H*	*H*	—	—
Dog	*H*	*H*	—	—
Guinea-pig	*H*	*H*	—	—
Mouse	*H*	*I*	*H*	*I*
Human	*H*	*I*	*H*	*I*

H, 90–100 per cent hydrolysis of substrate; *I*, 90–100 per cent inhibition; —, no hydrolysis of substrate.

Table 1 indicates that though all the sera tested hydrolysed butyrylcholine, only mouse and human sera hydrolysed dihydromurexine under the conditions of these experiments. Moreover, only mouse and human serum cholinesterases were inhibited by lysergic acid diethylamide. Similar findings were obtained employing BOL as the inhibitor.

Further experiments have shown that both mouse and rat brain homogenates hydrolyse butyrylcholine. However, the pseudocholinesterase of mouse brain differed from that of rat brain in two respects: (1) mouse brain cholinesterase catalysed the hydrolysis of dihydromurexine, and (2) this enzyme was strongly inhibited by lysergic acid diethylamide or BOL. Rat brain cholinesterase did not display either of these properties. Thus, in all cases tested so far, a potent inhibition of cholinesterase by lysergic acid diethylamide or BOL was observed only when the enzyme was capable of hydrolysing dihydromurexine.

These results suggest that a specific enzyme–substrate relationship (ability of some cholinesterases to hydrolyse dihydromurexine) is the basis for the selectivity displayed by lysergic acid diethylamide and BOL towards various cholinesterases. The identification and evaluation of other choline esters may aid in clarifying this observation.

I. I. A. Tabachnick
M. E. Grelis

Department of Pharmacology,
Scientific Research Division,
Schering Corporation,
Bloomfield, New Jersey.
July 21.

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Radiometric Titrations with Complexons

Among the reactions commonly used in volumetric work, only two are suitable for radiometric titrations: (*a*) reactions which result in combinations which are only very slightly soluble[1,2]; (*b*) reactions which result in complexes[3].

At first, precipitation reactions were used, one of the reactants being labelled with a radioactive isotope. The precipitate resulting from titrations was separated from the solution by filtration or centrifugation and the equivalence point was determined by measuring the activity of the solution. Thus, halides were determined with radioactive silver; manganese, uranium, thorium, beryllium and zirconium with radioactive phosphate; thallium with tetraphenylboron, etc. (*loc. cit.*).

I. M. Korenman[3] and F. G. Duncan *et al.*[4] introduced complexing reactions into radiometric titrations. Zinc, mercury and cobalt were determined by using dithizone as a complexing agent. The reaction product was separated from the solution, using the solubility of dithizone and its complexes in carbon tetrachloride (extraction). The same authors also proposed a non-isotopic tracer method, which consists in adding a radioactive isotope of another element (for example, cobalt-60) which may react with the complexing or precipitating agent only after the quantitative reaction of the cation under study has taken place.

This procedure has great advantages because it permits the choice of a convenient radioactive isotope and the determination of many cations with the same radioactive isotopes.

We have devised a radiometric titration depending on complexing reactions; the equivalence point is found, using a radioactive indicator in heterogeneous phase. The following procedure was employed: a cation, M^{2+}, was titrated with a complexing agent A^{2-}, and a precipitate containing a radioactive cation ($M_i{}^*B$) was used as indicator (in the heterogeneous phase). The complexing agent A^{2-} reacts with the cation M^{2+}, giving the stable and soluble complex MA. Meanwhile, the activity of the solution remains the same and is determined by the cosmic background and the ions $M_i{}^{*2+}$, arising in the dissociation of the precipitate ($M_i{}^*B$), according to its solubility product $L_{M_i{}^*B}$. After the complete disappearance of M^{2+}, the excess of added complexing agent reacts with $M_i{}^{*2+}$, dissolving the precipitate $M_i{}^*B$; the activity of the solution increases with increasing amount of complexing agent A^{2-} added after the equivalence point is reached. Fig. 1 shows the plot of the activity of the solution versus amount of complexing agent A^{2-}. The region *ab* of the curve corresponds to the reaction:

$$M^{2+} + A^{2-} \rightarrow MA \qquad (1)$$

and the region *bc* to the reaction:

$$M_i{}^*B + A^{2-} \rightarrow M_i{}^*A + B^{2-} \qquad (2)$$

In order that these reactions occur as above, the following conditions must be fulfilled: (1) the MA complex must be much more stable than the $M_i{}^*A$ complex, that is, $pK_{MA} > pK_{M_i{}^*A}$; (2) the solubility product $L_{M_i{}^*B}$ of the indicator precipitate and the stability constant $K_{M_i{}^*A}$ of the $M_i{}^*A$ complex must be related in such a manner as to shift reaction 2 to right; (3) M^{2+} must not react with the anion

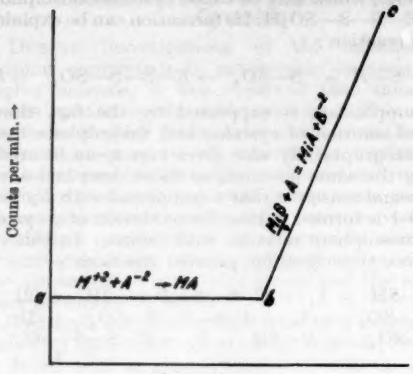

Fig. 1

B^{2-} of the labelled precipitate, in order to avoid taking the radioactive cation $M_i{}^{*2+}$ out of the combination.

The first system chosen for titrations was the following: complexon III was used as a complexing agent and radioactive silver as tracer (it gives a slightly stable complex with the complexing agent and may be used in the titration of many cations if condition 1 is fulfilled); silver has also a suitable half-life (270 days).

To get the radioactive tracer in the heterogeneous phase, radioactive silver was precipitated as Ag^*IO_3. The silver iodate is soluble, at convenient *p*H, in complexon III solutions according to condition 2. The titration of copper at a *p*H of approximately 9 will serve as an example. Fig. 2 shows the curves obtained when copper is titrated with complexon III using radioactive silver iodate as indicator in the heterogeneous phase. Curve 1 represents the titration of radioactive silver iodate with complexon without adding copper ions. Curves 2, 3 and 4 show the titration of different amounts of copper.

By this method a wide range of elements may be determined either by direct titration or by re-titration. Different complexing agents and radioactive indicators in the heterogeneous phase may be used, provided they fulfil the necessary conditions already mentioned.

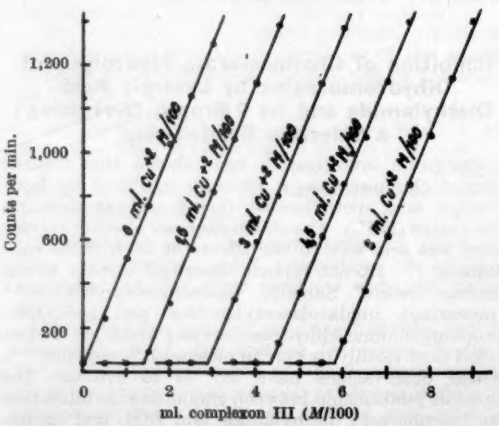

Fig. 2

In a paper to be submitted elsewhere the conditions in which a wide range of cations have been titrated in our laboratories will be described.

T. Braun
I. Maxim
I. Galateanu

Institute of Atomic Physics,
Casuta postală Nr. 35,
Bucharest.

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The Free Amino-Acids of the Cerebrospinal Fluid

Current literature contains confusing reports about the number and nature of the free amino-acids normally present in human cerebrospinal fluid[1]. Thus, while some investigators have detected only three to eight amino-acids[2], others have found as many as eighteen[3]. In many instances, a particular amino-acid was not present regularly, but was only observed in a few cases. For this reason, reports which suggest that certain amino-acids occur exclusively in certain diseases can only be regarded as speculative. The aim of the present work has been to establish whether or not a regular pattern of amino-acids exists in normal and abnormal cerebrospinal fluid.

Separation of the amino-acids of a number of samples of cerebrospinal fluid by high-voltage paper electrophoresis, using buffer solutions of different *p*H, led to the identification of between six and eight amino-acids in each sample. Two-dimensional paper chromatography gave better results, but with this method some amino-acids present only in trace amounts were sometimes insufficiently separated for clear identification. High-voltage paper electrophoresis was therefore combined with paper chromatography and the following standard procedure adopted: 5–10 ml. cerebrospinal fluid was deproteinized with acetone and then desalted electrolytically in a modified Astrup desalting apparatus provided with an outer glass vessel containing an ice/salt cooling mixture. Under these conditions, adequate desalting for the high-voltage paper electrophoresis and chromatographic separation could be obtained in 5–10 min. without destruction of the amino-acids. The desalted solution was then evaporated to dryness *in vacuo* and the residue dissolved in distilled water (1/100 volume of original cerebrospinal fluid). 5–10 μl. of this solution was applied to a Schleicher and Schüll 2043*a* paper (60 cm. × 28 cm.), and high-voltage paper electrophoresis was conducted for 75 min. at 80–100 V./cm., in a fumaric – acetic acid buffer, *p*H = 1·9, using a Werner and Westphal chamber[4]. The paper was then dried and run as an ascending chromatogram for 16 hr. with *n*-butanol/acetic acid/water (4 : 1 : 5) as solvent. It was developed with the ninhydrin reagent of Levy and Chung[5]. This technique enabled a clear separation and identification of most of the amino-acids in cerebrospinal fluid to be made. Identification of cysteine–cystine, methionine, phenylalanine, and proline was achieved by running one-dimensional chromatograms (using 30 μl. of solution = 3 ml. cerebrospinal fluid) which were then developed with specific reagents for these amino-acids.

A series of twenty cases was investigated using this method, and the following twenty-five substances were regularly found in the cerebrospinal fluid: alanine, α-amino-*n*-butyric acid, γ-amino-*n*-butyric acid, arginine, aspartic acid, cysteine–cystine, glutamine, glutamic acid, glycine, histidine, leucine-*iso*leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, valine and three unknown substances, two of them probably biogenic amines. Occasionally, two other substances were also found, but these disappeared after hydrolysis; their electrophoretic and chromatographic characteristics suggested that they may be peptides.

The clinical diagnoses of the cases examined included cerebral atrophy of varying etiology, postencephalitic parkinsonism, cerebrospinal syphilis, diabetes insipidus, idiopathic and symptomatic epilepsy, cerebrovascular diseases, other neurological and non-neurological diseases; some normal individuals were also included. There was no correlation between the electroencephalogram, air-encephalogram, usual routine laboratory tests of cerebrospinal fluids and blood and the spectra of the free amino-acids in the cerebrospinal fluid.

From these results it is concluded that the qualitative analysis of the free amino-acids in the cerebrospinal fluid is of no diagnostic value. The amino-acids are basic constituents of both normal and pathological cells, and it is therefore unlikely that diseases which affect only a small percentage of the total number of nerve cells will alter the spectrum of free amino-acids in the cerebrospinal fluid over and above the large quantitative variations reported in the literature.

H. G. Knauff

II Medical Clinic,
University of Munich,
Ziemssenstrasse 1, Munich.

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Oxidation of Ethyl Alcohol to Acetic Acid by Proliferating Yeasts

In certain typical Spanish wines (Moriles, Montilla, Jerez, etc.), a film is formed on the surface after fermentation.

From a red wine of Galicia (Spain) I have isolated a strain of *Saccharomyces* that is able to form a film ('flor') on red wine (Fig. 1). Inoculated into grape must with a 23 per cent sugar content, this culture produces a vigorous alcoholic fermentation, typical of *Saccharomyces*, and after fermentation forms a film.

In studying the characteristics of this strain, I found that in the yeast nitrogen base for the carbon-assimilation tests of Wickerham, with 3 per cent of ethyl alcohol a pellicle is formed after 8 days at 25° C., and acid is produced; after 32 days at 25° C. the *p*H was 2·6 and finally 7·5 ml. 0·1 *N* sodium hydroxide were needed to neutralize 10 ml. of medium. The acid was exclusively acetic acid.

Fig. 1. *Saccharomyces aceti* strain *VT* 12–1, film on red wine after 25 days at room temperature

The same basal medium with 1 per cent of methanol showed, after 24 days, a slight growth, but it remained neutral and only a thin incomplete pellicle was formed. The cells of this pellicle were almost exclusively asci with one, two, three and four smooth, round ascospores (Fig. 2). *iso*Propanol is also oxidatively assimilated, with the production of acetone.

The culture can ferment glucose only; it can assimilate galactose, sucrose, maltose and lactose, but the assimilation is weak and in every case a thin pellicle is formed in which there are many asci. In malt extract broth, no pellicle is formed in three days at 25° C.

This organism is considered to be a new species in the genus *Saccharomyces*. The name *Saccharomyces aceti* is suggested. I have isolated two other strains of this species.

From a white wine of Montánchez (Cáceres, Spain) I have isolated another *Saccharomyces* strain, which also forms a film on white wine and on grape must after alcoholic fermentation. This strain shows a great resemblance to *Saccharomyces aceti* in its oxidizing properties, but it ferments glucose, saccharose and raffinose 1/3. I have isolated two other strains with the same properties, except for their sugar assimilation: the first of these three assimilated galactose and lactose weakly, the second assimilated maltose weakly, and the third did not assimilate galactose, maltose or lactose. I did not consider these differences important enough to differentiate these strains from the other one. These organisms are considered to be a new species in the genus *Saccharomyces*. The name *Saccharomyces oxidans* is suggested.

Cultures of *Saccharomyces aceti* and of *S. oxidans* have been deposited in the Yeast Collection of the Central Bureau voor Schimmelcultures in Delft.

A detailed account of this work, which was supported by a grant of the March Foundation, will be published elsewhere.

Juan Santa María

Sección de Bioquímica,
Instituto Nacional de
Investigaciones Agronómicas,
Madrid. July 18.

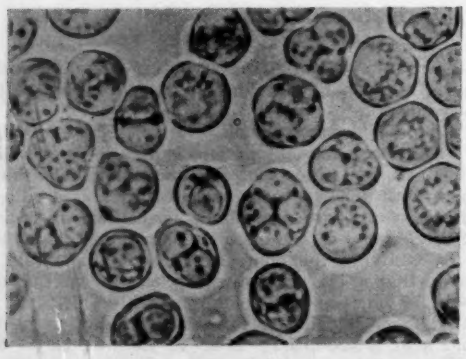

Fig. 2. *Saccharomyces aceti* strain *VT* 19–1. Asci with one, two, three and four spores. From the yeast nitrogen base of Wickerham with 1 per cent methanol, after 24 days at 25° C. Unstained. (× 1,750)

Analeptic Action of Anibine

Anibine, the alkaloid from the wood of the South American rosewood trees (*Aniba Duckei* Kostermans and *Aniba rosaeodora* Ducke), has been shown to be 4-methoxy-6-(3′-pyridyl)-α-pyrone[1].

Investigating the pharmacological properties of this substance, we found its action to be similar to that of nikethamide, with which it also shares certain structural features. In the present communication we wish to report on the analeptic action of anibine and compare its toxicity with that of nikethamide.

Solutions of both substances of equal concentration and acidity were used in the tests. Nikethamide is miscible with water. The solubility of anibine or even of its hydrochloride in water is small at room temperature. Solutions of 2 per cent anibine adjusted to *p*H 3·5 with hydrochloric acid, prepared by heating, were stable for some time at 37°. Distilled water, physiological salt solution or isotonic glucose solution were used indifferently as diluents without affecting the results of the tests.

Anibine and nikethamide produced the same toxic symptoms in albino mice of 20 gm. weight when 1 ml. of the 2 per cent solutions (20 mgm.) was injected. Intravenous injections resulted in immediate clonic convulsions, congestion and nasal hæmorrhage followed by death. These symptoms were somewhat delayed if the injections were applied intraperitoneally. Intravenous injections of 0·5 ml. (10 mgm.) produced the same symptoms, even if at a slower rate, death following after 20–25 min. Intraperitoneal injections of 0·5 ml. of the 2 per cent solutions showed the difference in toxicity of the two substances. Animals injected with the solution of nikethamide suffered prolonged convulsions without lethal result during 1 hr.; animals injected with the solution of anibine did not show any disturbance.

Tests of analeptic action were carried out on male rabbits, weighing approximately 2 kgm., using solu-

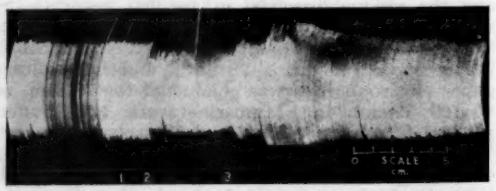

Fig. 1. Respiration record (pressure variation at the trachea) of a male rabbit, weighing 1·8 kgm., which remained without anæsthesia during the test (1 cm. on the abscissa = 1 min.). (1) Respiration-rate at the start, 100 per min. (2) Jugular injection of 12 mgm. of morphine hydrochloride: the respiration-rate fell to 75 per min. and the amplitude of breathing diminished. (3) Jugular injection of 20 mgm. of anibine in 1 ml. of isotonic glucose solution at 37°: the rate rose to 120 per min. and the amplitude increased

tions of different concentrations and recording graphically the effect upon breathing and arterial pressure. Intravenous injections of 0·5 ml. of 0·1 and 0·2 per cent solutions of anibine reduced slightly the arterial pressure. Higher doses (1 ml. of 2 per cent solution of anibine) produced a marked analeptic effect on animals the respiratory centres of which had been previously depressed by morphine (Fig. 1). Blank tests in which only the diluents, at the same *p*H, were injected failed to affect respiration.

One of us (O. R. G.) is indebted to the Conselho Nacional de Pesquisas, Brazil, for financial aid.

N. Botafogo Gonçalves
Joao Canali Corrêa Fº.

Instituto Osvaldo Cruz,
Ministério da Saúde.

Otto Richard Gottlieb

Instituto de Química Agrícola,
Ministério da Agricultura,
Rio de Janeiro, Brazil.

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Indole-3-aldehyde from Tissues

Salhanick and Berliner[1] studied a feminizing adrenal tumour from a man of 22. It weighed 920 gm., of which 523 gm. were used for chemical extraction. The frozen tissue was homogenized and extracted with acetone, leaving a 'protein' residue. The aqueous acetone was extracted with chloroform and the resulting lipid was partitioned between hexane and 75 per cent methanol. The latter was reduced to small bulk and extracted with chloroform to yield 0·67 gm. of yellow oil. The protein residue was digested with alkali (1 l. 5 per cent sodium hydroxide) for two days and extracted first with ether at *p*H 10 and then at *p*H 3 after acidification. After partitioning the extract, 0·26 gm. of oil was obtained. Chromatography on silica gel yielded progesterone, equilenin and two unidentified materials designated *AAE-I-BI* (44 μgm.) and *AAE-I-AI* (23 μgm.) from the acetone extract. From the protein hydrolysate extract the same materials were obtained in somewhat larger amounts (*AAE-I-AI*, 109 μgm.) together with three further unidentified fractions.

Substance *AAE-I-AI* showed (in methanol) absorption peaks at 242, 260 and 295 mμ and in concentrated sulphuric acid peaks at 240, 265 and 330 mμ. The published absorption curves show well-marked patterns of maxima and minima. The preparation exhibited a positive Millon test, regarded as indicative of a 3 : 5-unsubstituted hydroxyphenyl group; it also gave a positive Zimmermann reaction for 17-ketosteroid. The total amount of material (132 μgm.) was very small, and there was no evidence as to purity. It was uncertain whether the chemical tests and the spectrophotometric characteristics were due to a single compound.

There can now, however, be little doubt that the spectroscopically recognizable constituent of *AAE-I-AI* was indole-3-aldehyde (Table 1) or a chromophorically identical substance.

The most surprising feature of the properties of the aldehyde is the change in absorption spectra in the presence of alkali.

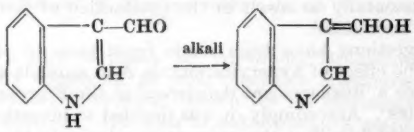
The indole nucleus shows an intense absorption band near 220–225 mμ and a region of weaker selective absorption around 270–280 mμ (order 10,000) with some fine structure. This applies to indole, 2-methyl-, 3-methyl- and 7-methyl-indole as well as to indole-3-acetic acid and tryptophan. Addition of sodium ethoxide to ethanolic solutions effects no marked change.

Table 1. Properties of Indole-3-aldehyde (Formylindole)

Solvent*	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$	$\lambda_{min.}$ (mμ)	$\varepsilon_{min.}$
Ethanol	242	13,700	227	4,700
	260	11,700	252	11,000
	296	13,440	272	7,200
Ethanolic sodium ethoxide (0·1 *M*)	266	19,900	237	2,500
	326	20,400	293	5,850
Concentrated sulphuric acid	235	28,800	220	17,900
	266	54,200	250	28,700
	329	46,700	285	10,100

* See also G. F. Smith (ref. 2) for aqueous and aqueous alkaline solutions.

The new effect with indole-3-aldehyde has been attributed to the change:



In fact, we have found that there is an equilibrium which varies with the *p*H.

Indole-3-aldehyde has also been shown[3] to be one of the photochemical products of the irradiation of indole-3-acetic acid with light of wave-length 280 mμ.

In the course of an extended study of tissue unsaponifiable fractions we have noted a material (fraction *SF*) showing $\lambda_{max.}$ 235 and 282 mμ (in *cyclo*hexane) and $\lambda_{max.}$ 242 and 295 mμ (inflexion 260 mμ in ethanol). Among the best sources of this material were pancreas and spleen from several species. By chromatography on alumina the *E*(1 per cent, 1 cm., 282 mμ) value was raised to 50 and at that stage a small quantity was subjected to molecular distillation and yielded a few mgm. of white crystals. The absorption curves for this preparation in ethanol and in sulphuric acid reproduced those of *AAE-I-AI* but the chemical constitution remained unknown. The crystals gave a negative Millon reaction and a positive Tollens reaction. Small amounts of molecular distillates were kindly examined for us in the mass spectrograph at the Thornton Research Laboratories of Shell, Ltd. Mr. A. Quayle and his colleagues (to whom we are much indebted) found in the purer preparation evidence that the largest particles were of mass number (molecular weight) 144 and 145 and with nitrogen present it was suggested that an indole derivative was possible. Examination of authentic indole-3-aldehyde soon confirmed the nature of the selectively absorbing substance. It is perhaps too early to discuss in detail the significance of the detection of indole-3-aldehyde in the adrenal tumour, in normal pancreas and spleen and in the photoproducts of indole-3-acetic acid. The idea that it is a straightforward artefact derived from the tryptophan of proteins is not consistent with the evidence.

R. A. Morton
N. I. Fahmy

Department of Biochemistry,
University of Liverpool.
July 24.

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Effect of Vitamin B Complex on the Teratogenic Activity of Hypervitaminosis A

Some years ago, attention was directed by Cohlan[1] to the teratogenic effects of large doses of vitamin A administered to laboratory animals during pregnancy. His observations have since been fully confirmed by a number of workers.

Recently, it has been shown that the teratogenic activity of hypervitaminosis A can be modified by the concurrent administration of other substances. In particular, it has been observed that cortisone and 4-methyl-2-thiouracil augment or potentiate the teratogenic action, whereas insulin tends to diminish it[2].

Among the commonest malformations of the young induced by maternal hypervitaminosis A are cleft palate and deformities of the brain and skull such as exencephaly and hydrocephaly. Maternal deficiencies in vitamins of the B complex have also been shown experimentally to result in the production of similar fœtal anomalies[3].

Suggestions have been made from time to time that the effect of hypervitaminosis A in animals is to produce a 'displacement deficiency' in the B group of vitamins[4]. Accordingly, it was decided to investigate the possibility that the concurrent administration of the B vitamins in large doses might mitigate the teratogenic effects of hypervitaminosis A. In the experiments reported here a 'blunderbuss' injection of B vitamins was employed as a preliminary to an examination of the effects of individual vitamins of the B group.

Female rats of the Wistar strain were used as in the experiments reported previously. Vitamin D (2,000 I.U.) and 50 mgm. vitamin E were given by mouth at the beginning of the experiment. After mating with normal male rats, pregnancy was determined by the presence of spermatozoa in the vagina. One group of pregnant female rats received 40,000 I.U. vitamin A acetate daily by gastric intubation from the eighth to thirteenth days of pregnancy. A second group received 40,000 I.U. vitamin A daily and, in addition, 0·5 ml. vitamin B complex by subcutaneous injection from the eighth to thirteenth days. The B injection contained aneurine hydrochloride 25 mgm., nicotinamide 16 mgm., riboflavin 0·4 mgm., pyridoxine hydrochloride 5 mgm. in benzyl alcohol, 14 mgm., as a vehicle.

The rats were killed on the twentieth day of pregnancy and the young removed for examination. Only gross deformities involving the brain or palate were considered.

In the group of rats which received vitamin A alone, 9 young out of 121 showed deformities of the brain, an incidence of 7·4 per cent. No young with deformities of the brain were encountered among the young from the groups which received both vitamin A and vitamin B complex. The results of the experiment in relation to malformations of the palate are shown in Table 1. It will be noted that when vitamin A alone was given, 31·4 per cent of the young showed clefts of the palate, whereas when vitamins of the B group were given in addition only one of the young had a cleft palate, an incidence of 1·5 per cent.

It seems reasonable from these experiments to reach the tentative conclusion that the failure of vitamin A to produce a comparable incidence of malformations in the two groups was due to the presence, in the second group of animals, of a large amount of vitamins of the B group. Further experiments are in progress with the view of determining whether the protective effect requires the presence of a number of B vitamins or is mediated by a single member of the group.

Table 1

Treatment	No. of rats	Total No. of young	No. of young with cleft palate	Percentage of young with cleft palate
Vitamin A	16	121	38	31·4
Vitamin A Vitamin B	9	65	1	1·5

The hypothesis that it is the relative balance between the amounts of different vitamins which is critical for normal development receives some additional support from these experiments. Furthermore, the results are not discordant with the view that an excess of vitamin A may produce a degree of vitamin B deficiency which can be compensated by the concurrent administration of this vitamin.

This work was supported by a grant from the Nuffield Foundation which is gratefully acknowledged.

J. W. Millen
D. H. M. Woollam

Department of Anatomy,
University of Cambridge.
July 29.

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Ribonuclease and Deoxyribonuclease Activities during Normal and Neoplastic Growth

A relationship has been repeatedly demonstrated between the rate of growth of a tissue and its ribonuclease (RNase) and deoxyribonuclease (DNase) activities[1–4].

These findings stimulated the present investigation. The nucleic acid content and nuclease activities of a number of human and animal malignant tumours have been determined. The determinations were carried out as described earlier[1,2]. Except in the case of the Ehrlich ascites tumour the tissues of origin have also been examined.

The results are summarized in Table 1. As in previous papers[1–3] enzyme activities are expressed relative to deoxyribonucleic acid–phosphorus content. In some tumours the ribonuclease activity was equal to that in the tissue of origin; in others it was lower. Only in pancreatic carcinoma was there an increase. Ledoux *et al.*[5] have recently reported that in human cervical cancer the ratio of acid ribonuclease activity to ribonucleic acid content is lower than in the normal tissue of origin. Accordingly, the corresponding ratios have been calculated for the tissues examined in the present experiments (Table 1). Two of the tumours gave results which support Ledoux's concept: the Ehrlich ascites carcinoma, which contained no measurable ribonuclease; and one case of stomach carcinoma in which the ribonuclease/ribonucleic acid ratio, although high, was significantly lower than the ratio given by the gastric mucosa of the same patient.

Table 1. Nucleic Acid Content and Nuclease Activities in Normal and Neoplastic Tissues

Tissue	μgm. RNAP/mgm. dry weight	μgm. DNAP/mgm. dry weight	RNAP/DNAP	RNase/μgm. DNAP*	RNase/μgm. RNAP*	DNase/μgm. DNAP†
Human :						
Gastric mucosa	1·91	1·21	1·58	24·1	15·3	1·98
Cancer of the stomach	3·55	2·14	1·65	26·8	16·3	2·39
Gastric mucosa	1·42	0·76	1·88	97·0	51·7	2·71
Cancer of the stomach	1·94	1·36	1·43	49·2	34·4	2·68
Liver	10·65	2·84	3·76	5·5	1·5	8·35
Cancer of the liver	1·41	1·14	1·24	5·9	4·8	2·32
Pancreas	12·31	1·03	11·91	2·3	0·19	3·10
Cancer of the pancreas	3·26	0·42	7·72	5·5	0·71	4·02
Animal :						
Mammary gland, pregnant rat	0·92	0·29	3·17	0·39	0·13	5·6
DMBA tumour‡	4·89	3·65	1·34	0·19	0·15	6·0
Ehrlich carcinoma, ascitic form	9·63	3·18	3·03	No measurable activity	—	0·7

* Ribonuclease activities determined by incubating tissue breis with ribonucleic acid at *p*H 7·70, adding trichloracetic acid–lanthanum acetate, centrifuging and reading the optical density in the supernatant against the appropriate blank. The activities are expressed as optical density increase/hr./μgm. tissue deoxyribonucleic acid–phosphorus or ribonucleic acid–phosphorus (DNAP or RNAP).

† Deoxyribonuclease activities determined by incubating tissue breis with deoxyribonucleic acid at *p*H 5·25 and following the procedure above. The increase in deoxypentose in the supernatant was determined colorimetrically and from this the amount of deoxyribonucleic acid–phosphorus was calculated. The deoxyribonuclease activities are expressed as deoxyribonucleic acid–phosphorus liberated per hr./μgm. tissue deoxyribonucleic acid–phosphorus.

‡ Dimethylbenzanthracene tumour. These have been studied by Scholler *et al.* (ref. 8). They were obtained through the courtesy of Dr. Jean Scholler.

In the other tumours the ratio was equal to or greater than that of the tissue of origin. It therefore appears that a diminished alkaline ribonuclease/ribonucleic acid ratio is not characteristic of all tumours.

In general, the deoxyribonuclease activities of the neoplastic tissues studied did not differ greatly from those of the tissue of origin. The only exception was a hepatic carcinoma which had a deoxyribonuclease activity only 25–30 per cent that of liver.

Although the growth-rates of neoplastic tissues differ within wide limits, they usually grow faster than their tissues of origin. The incorporation of isotopes into various cellular constituents, in particular into the nucleic acids, also occurs at higher rates in tumours than in their tissues of origin[6]. In this respect there is a similarity between rapidly growing non-malignant and neoplastic tissues. The synthetic activities as reflected by the rate of isotope incorporation are apparently stimulated in both types of growth. The ribonuclease and deoxyribonuclease activities, on the other hand, show completely different patterns in normal and neoplastic growth. Whereas the normal tissue response to a growth stimulus involves a sharp increase in the activities of these enzymes, neoplastic tissues do not seem to respond in this way.

There has been some speculation on the role of the nucleases in the synthesis or degradation of nucleic acids *in vivo*. There is a positive correlation between the activities of these enzymes and the rate of isotope incorporation into the nucleic acids during the non-malignant growth[4] in regenerating liver. This suggests a synthetic activity. The lack of correlation, however, which is found during neoplastic growth argues against this interpretation. It is more likely that they have a degradative or regulatory function. The parallelism in normal growth between nuclease activities and the rate of isotope incorporation into the nucleic acids contrasts sharply with the absence of such a relationship during neoplastic growth. During normal growth the synthetic activities[7] seem counterbalanced by the ribonuclease and deoxyribonuclease activities, whereas in neoplastic growth there appears to be an imbalance between these systems. The events during carcinogenesis leading to this state are now the subject of investigation in this laboratory and a report will shortly be published.

We wish to thank Dr. Alexander Brunschwig for kindly supplying us with operative specimens, and Dr. George Bosworth Brown for his encouragement and interest. We wish to acknowledge a grant from the American Cancer Society and the Swedish Cancer Society to Dr. Sam Brody. This investigation was supported in part by funds from the American Cancer Society, National Cancer Institute, National Institutes of Health, Public Health Service (Grant *CY*–3190), and from the Atomic Energy Commission (Contract No. *AT*(30–1)–910).

Sam Brody*
M. Earl Balis
Laboratories of the Sloan-Kettering Division,
Cornell University Medical College,
New York 21.

* Permanent address : Department of Women's Diseases, Karolinska Sjukhuset, Stockholm 60, Sweden.

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Physical Reactivity of Liver and Brain Cortex Mitochondria

In recent investigations[1] we found that rat brain cortex mitochondria, isolated in 0·25 *M* sucrose by method II of Brody and Bain[2], had, in the same experimental conditions, certain physical properties different from those of rat liver mitochondria isolated by the method of Myers and Slater[3]. The incorporation of 0·01 *M* ethylenediaminetetraacetate in the 0·25 *M* sucrose, used by Slater[4] in the preparation and stabilization of heart sarcosomes, has yielded some results concerning the apparent stability of brain granules at 25° C. The ethylenediaminetetraacetate used was the disodium salt, brought to *p*H 7·5 with potassium hydroxide, and the solution was made up with de-ionized water. Experiments were performed with mitochondria isolated from the liver and the brain cortex of normal white rats (Wistar strain), three months old. The tissues were

Table 1. Rate of Variation in Optical Density of Suspensions of Liver and Brain Cortex Mitochondria obtained at 25° C., in 1-cm. Cells at 550 mμ. The Zero-Time Value was obtained by Extrapolation and was Approximately 0·3. Each Percentage Number represents the Mean Value of Four Experiments

Condition	Other additions	Percentage variation of optical density					
		Liver			Brain cortex		
		2 min.	6 min.	10 min.	2 min.	6 min.	10 min.
Mitochondria isolated in 0·25 *M* sucrose							
0·05 *M* sucrose 0·01 *M tris* pH 7·4	None	+ 6	0	− 21	− 6	− 10	− 9
	10^{-3} *M* $CaCl_2$*	+ 12	+ 12	+ 3	+ 1	+ 2	+ 3
	10^{-3} *M* $MgCl_2$	+ 4	+ 7	+ 7	+ 1	− 1	− 1
	5×10^{-3} *M* $MgCl_2$				+ 5	+ 8	+ 10
	10^{-3} *M* $Na_2HPO_4.2H_2O$	− 28	− 32	− 33	− 6	− 9	− 9
	10^{-3} *M* ATP	+ 5	+ 9	+ 10	− 6	− 9	− 11
Mitochondria isolated in 0·25 *M* sucrose + 0·01 *M* ethylenediaminetetraacetate							
0·05 *M* sucrose 0·01 *M tris* pH 7·4	None	− 3	− 4	− 4	− 11	− 18	− 20
	10^{-3} *M* $CaCl_2$	+ 6	− 4	− 16	+ 2	+ 2	0
	10^{-3} *M* $MgCl_2$	− 1	0	+ 2	− 2	− 3	− 3
	5×10^{-3} *M* $NaH_2PO_4.2H_2O$	+ 4	+ 6	+ 7	− 5	− 8	− 9
	10^{-2} *M* $NaH_2PO_4.2H_2O$				− 2	− 2	− 3
	10^{-3} *M* ATP				− 14	− 19	− 22
	10^{-5} *M* $CoCl_2$	− 3	− 2	0	− 12	− 17	− 20
	10^{-5} *M* $HgCl_2$	0	+ 2	+ 4	− 9	− 13	− 15
	10^{-5} *M* $Ag(NO_3)_2$				− 9	− 12	− 14

* The percentage number was − 27 at 16 min.

rapidly homogenized in a Potter–Elvehjem homogenizer fitted with a 'Teflon' pestle, with ten volumes of the sucrose–ethylenediaminetetraacetate medium. Nuclei, erythrocytes, intact cells and debris were sedimented by centrifugation at 700*g* for 10 min., and the mitochondria then separated by centrifugation of the supernatant fluid at 8,500*g* for 10 min. (rate controlled by an electronic stroboscope, Philips *PR* 9103). The mitochondria were washed by resuspension and resedimentation in the same conditions. The final pellets were suspended in the sucrose–ethylenediaminetetraacetate medium, so that the mitochondria from 0·80 gm. of fresh liver or from 0·90 gm. of fresh brain cortex were suspended in 1·0 ml. All operations were carried out at 0° C. The purity of isolated fractions was controlled morphologically with phase microscopy and chemically by the colorimetric test of Potter, Recknagel and Hurlbert[5] (reduction of janus green *B* to yield diethylsafranine at 37° C.). The mitochondria were used immediately following their isolation. Mitochondrial volume changes were studied at 25° C. by following the optical density of suspensions at 550 mμ, in 1-cm. cells, with a Meunier photometer (Jobin and Yvon, Arcueil), as described by Cleland[6]. The basic test system (volume 7 ml.) contained mitochondria (about 0·03 ml. of the stock suspension, giving an initial optical density of approximately 0·300) suspended in 0·05 *M* sucrose and 0·01 *M tris* (hydroxymethyl) aminomethane, *p*H 7·4; the solution was made up in de-ionized water. The first reading was taken 30 sec. after the addition of the mitochondria and subsequent readings at 2-min. intervals for 10 min.; the zero-time value was obtained by extrapolation.

Table 1 and Fig. 1 show that the addition of 0·01 *M* ethylenediaminetetraacetate to the isotonic sucrose medium during the preparation of mitochondria provided liver granules which were very stable when tested in the hypotonic sucrose medium, in complete agreement with earlier results[7]; the brain granules, however, swelled rapidly. On the other hand, the swelling of brain mitochondria was largely prevented by calcium ions; in contrast, these ions at the same concentration (10^{-3} *M*) produced rapid swelling in liver mitochondria. Magnesium ions also prevented the swelling of brain granules, but had no effect on the stability of liver granules.

As pointed out by Slater[4], it might be expected that the addition of the chelating agent ethylenediaminetetraacetate to the isolation medium would remove essential metals from the mitochondria, as well as prevent the binding of calcium ions by the granules. Christie, Judah and Rees[8] concluded that cobalt activates and stabilizes respiration in brain mitochondria treated with certain chelating agents (but not ethylenediaminetetraacetate). Table 1

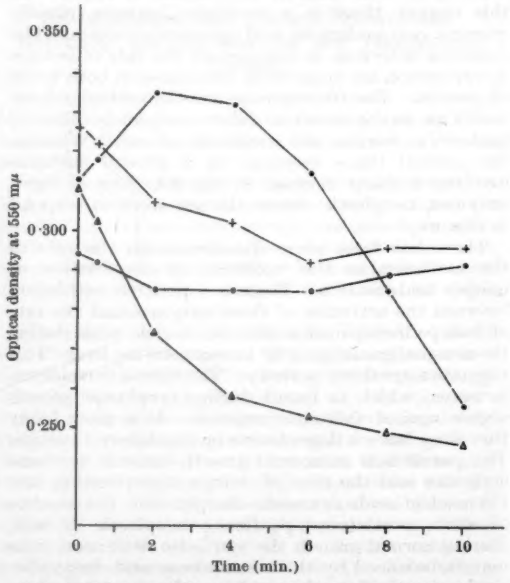

Fig. 1. Influence of the method of isolation on the degree of swelling of rat liver and brain cortex mitochondria. The test medium contained 0·05 *M* sucrose and 0·01 *M tris* buffer *p*H 7·4, at 25° C. Liver (●—●) and brain cortex (+—+) mitochondria isolated in 0·25 *M* sucrose. Liver (○—○) and brain cortex (△—△) mitochondria isolated in 0·25 *M* sucrose + 0·01 *M* ethylenediaminetetraacetate

shows that cobalt ions (10^{-5} *M*) did not affect the swelling of brain granules. On the other hand, Slater[4] supposed that ethylenediaminetetraacetate would also activate sensitive —SH enzymes by removing contaminating heavy metals. In Table 1 we see that mercuric and argentic (Ag++) ions had no effect on the swelling of brain mitochondria; mercuric ions had a slight preventive effect on liver mitochondria.

Hunter and Ford[7] considered the protective effect of ethylenediaminetetraacetate against pre-incubation of liver mitochondria with phosphate to be completely unexpected. Our results (Table 1) confirm earlier ones but in contrast phosphate had a slight preventive action on the swelling of brain mitochondria. Numerous authors[4,7,9] established that adenosine triphosphate had a strong preventive effect on the swelling of liver mitochondria, heart sarcosomes and cytoplasmic granules of diverse tissues. In this paper we confirm our earlier results[1]: adenosine triphosphate, at a concentration of 10^{-3} *M*, had no preventive effect on the swelling of brain cortex mitochondria isolated in the sucrose–ethylenediaminetetraacetate medium.

Berger[10] concluded that the different sensitivity of the mitochondria of liver and brain tissues towards chlorpromazine is determined rather by the structural differences than by some intrinsic differences in metabolic pattern: brain mitochondria has a greater adsorption capacity than liver mitochondria. This capacity was confirmed recently by Kunz[11], who has shown that, in contrast to rat liver mitochondria, brain granules do not require the addition of magnesium ions for the oxidative decarboxylation of pyruvate. Our preliminary results support the view that the so-called membrane of the brain mitochondria possesses a specific structure which confers a high physical stability on these granules.

This work will be continued and will be published in full elsewhere.

J. Gayet

Laboratoire de Physiologie Générale,
Faculté des Sciences,
Université de Nancy. July 10.

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Identification of Meprobamate by Adsorption Chromatography on Chromatoplates

Methods have recently been proposed (refs. 1 and 2 and Walkenstein, S., Knebel, C., MacMullen, J., and Seifter, J., private communication) for the identification and quantitative determination of 2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate (meprobamate) in blood and urine. These methods are sensitive and practical but appear to be insufficiently specific for use in analytical toxicology.

Paper chromatography was employed by Riebeling[3] for the detection of meprobamate combined with glycuronic acid in urine, but only the glycuronic acid is in fact detected by this method. Applications of paper chromatography to the separation and identification of meprobamate are very difficult because the compound is insoluble in water and because there are no methods for detecting the spots.

We have successfully employed a method for identifying meprobamate, based on adsorption chromatography on chromatoplates.

Chromatoplates were prepared using Demole's[4] modification of Reitsema's method[5]. 54 ml. of distilled water is added to a mixture of 28·5 gm. of pure silicic acid and 1·5 gm. of rice starch. The suspension is heated over a flame almost to boiling point to obtain a thick paste which is then diluted with 20–30 ml. of water and again heated to the same temperature for some minutes. After cooling, 10 ml. of the suspension is poured into the centre of a glass plate 17 cm. × 11 cm. × 0·2 cm. The liquid is quickly distributed on the surface with a spatula, and the layer is made homogeneous by rapid transverse movements of the plate.

Chromatoplates are left to dry for a few hours at room temperature and are then activated by heating in an oven for 30 min. at 100–105° C. The chromatoplates so prepared are stored in desiccators with anhydrous calcium chloride.

Meprobamate is isolated from urine by adding 0·2 ml. of *N* sodium hydroxide and 10 ml. of ether to 5 ml. of urine in a glass-stoppered centrifuge tube.

After shaking, the tube is centrifuged, the ether is decanted, and 60 mgm. of charcoal is added. This mixture is again shaken, filtered and evaporated to dryness under a stream of air. The dry residue is dissolved in 0·1 ml. of ethyl alcohol and known amounts of the alcoholic solution are quickly spotted on the starting line of the chromatoplate under a stream of hot air. The spotting of the plates inside the desiccator, as suggested by Demole[4], was found to be unnecessary for routine work.

Ascending development is carried out in a glass jar with cyclohexane and absolute ethyl alcohol (85 : 15) as solvent. After development for about 2 hr., when the solvent is 10–11 cm. from the starting line, the chromatoplate is removed, dried at room temperature and sprayed lightly with concen-

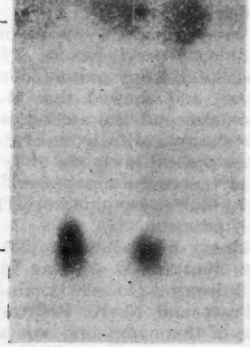

Fig. 1

trated sulphuric acid. A suitable sprayer bottle is described by Kirchner *et al.*[6]. The plate is then heated in an oven at 110–115° C. for 2–3 min., sprayed lightly with distilled water and reheated as before for some minutes, until yellow spots of meprobamate appear. Overheating should be avoided to prevent darkening of the chromatoplates. The spots, which disappear in about a day, have R_F 0·30. Exact R_F values are obtained only in the central zone of the chromatograms; at the periphery of the plates R_F values are higher, due to the border effect.

Unidentified impurities migrate with the solvent front (Fig. 1) and become violet under the sulphuric acid treatment.

A quantitative determination can be easily made by cutting out the spot and transferring it to a test tube. 1 ml. of distilled water is added, and then after careful shaking, 1 ml. of a 0·2 per cent hydroquinone in concentrated sulphuric acid[3]. The test tube is then heated in a boiling water bath for 20 min., till a yellow colour is obtained; this has an absorption maximum at 420 mμ and can therefore be determined spectrophotometrically.

Further details will be published elsewhere.

Angelo Fiori
Mario Marigo

Istituto di Medicina Legale e delle Assicurazioni,
Università di Padova.
July 7.

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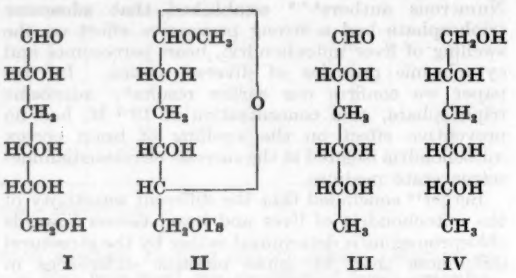
Synthesis of 3,6-Dideoxy-D-*ribo*-hexose (3,6-Dideoxy-D-glucose) and its Identification with Paratose

3,6-Dideoxy-hexoses are a new class of natural sugars; five members of this group are known at present: abequose[1], tyvelose[1,2], paratose[3] and colitose[4] have been found in specific polysaccharides extracted from various species of *Salmonella*, and ascarylose is obtained by hydrolysis of three glycolipids (the ascarosides *A*, *B* and *C*) extracted from the egg membrane and the cuticle of *Parascaris equorum*[5]. We have shown that all these sugars are 3,6-dideoxy-hexoses[6,7].

In a recent paper[8], we reported the synthesis of 3,6-dideoxy-D-*xylo*-hexose (3,6-dideoxy-D-galactose) and 3,6-dideoxy-D-*arabino*-hexose (3,6-dideoxy-D-mannose) and showed that the first is identical with abequose and the second with tyvelose, while colitose is the optical antipode of abequose and ascarylose is the optical antipode of tyvelose.

We now report the synthesis of 3,6-dideoxy-D-*ribo*-hexose (3,6-dideoxy-D-glucose) (III) and its identification with paratose.

3,6-Dideoxy-D-*ribo*-hexose (3,6-dideoxy-D-glucose) (III) was synthesized starting from 3-deoxy-D-*ribo*-hexose (3-deoxy-D-glucose) (I) kindly supplied by Drs. J. W. Pratt and N. K. Richtmyer[9]; the methyl-glucoside of this compound was tosylated, and after chromatography on magnesium silicate the methyl 3-deoxy-6-tosyl-D-*ribo*-hexoside (II) was obtained as a colourless syrup which was reduced by lithium aluminium hydride in a mixture of ether and benzene (1 : 1). After the usual treatment the methyl 3,6-dideoxy-D-*ribo*-hexoside was extracted with chloroform, the extract hydrolysed with 0·1 *N* hydrochloric acid in boiling water, and the free sugar then chromatographed on a column of Whatman cellulose powder. After elution of the sugar (II) with acetone/water (95 : 5) it was distilled at 0·05 mm. and obtained as a colourless syrup, $[\alpha]_D + 10 \pm 2°$; R_F 0·71 in butanol/pyridine/water (6 : 4 : 3); calculated for $C_6H_{12}O_4$: C, 48·64; H, 8·16 per cent; found: C, 48·68; H, 8·23 per cent.



I II III IV

The corresponding hexitol (IV) was prepared by reduction with sodium borohydride in water and obtained as crystals after distillation at 0·05 mm. After recrystallization from acetone, 3,6-dideoxy-D-*ribo*-hexitol (IV) was obtained as colourless needles, melting at 67–68°; $[\alpha]_D$ −18 ± 2°; R_F 0·53 in butanol/pyridine/water (6 : 4 : 3); calculated for $C_6H_{14}O_4$: C, 47·98; H, 9·40 per cent; found: C, 47·93; H, 9·68 per cent.

Paratose, which has recently been isolated by Davies *et al.*[3] from a polysaccharide of *Salmonella paratyphi A*, is a syrup, $[\alpha]_D + 8 \pm 2°$, and the hexitol (paratitol) obtained by reduction with sodium borohydride melts at 66–68° and has $[\alpha]_D - 16 \pm 2°$. (Found: C, 47·61; H, 9·52 per cent.)

The mixed melting point of paratitol and 3,6-dideoxy-D-*ribo*-hexitol (3,6-dideoxy-D-sorbitol) (IV) gave no depression. R_F values and infra-red spectra of both paratose and the crystalline hexitol are identical with those of the respective synthetic compounds.

C. Fouquey
J. Polonsky
E. Lederer

Institut de Biologie physico-chimique, Paris.

O. Westphal
O. Lüderitz

Dr. A. Wander Forschungsinstitut, Freiburg-Zähringen.

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Beta-globulin Polymorphism in Cattle, Sheep and Goats

Detection by paper electrophoresis. Using starch gel electrophoresis, six cattle[1], fourteen sheep[2], three goat (see later) and three human[3,4] β-globulin phenotypes have been recognized and indicated to be under the genetic control of three, five, two and three allelomorphs respectively. As one of us had noticed a variation in the number and position of the β-globulin zones in paper electrophoresis of animal sera, the two methods have been compared on sera from cattle, sheep and goats. Starch gel electrophoresis was carried out in phosphate buffer[1] *p*H 7·6, paper electrophoresis on a wide strip of horizontally suspended Whatman 3*MM* paper in barbiturate buffer *p*H 8·6, *I* 0·05. Typical examples of corresponding electropherograms of sheep and cattle sera are shown in Figs. 1 and 2. The electropherograms of goat sera shown in Fig. 3 are discussed below.

Table 1. Observed and Expected Distribution of Offspring Phenotypes from Goat β-Globulin Matings

Mating	Offspring $\beta^A\beta^A$ Obs.	Exp.	$\beta^A\beta^B$ Obs.	Exp.	$\beta^B\beta^B$ Obs.	Exp.
$\beta^A\beta^A \times \beta^A\beta^A$	No matings					
$\beta^A\beta^A \times \beta^A\beta^B$	8	8·5	9	8·5	0	0
$\beta^A\beta^A \times \beta^B\beta^B$	0	0	9	9	0	0
$\beta^A\beta^B \times \beta^A\beta^B$	1	0·75	1	1·5	1	0·75
$\beta^A\beta^B \times \beta^B\beta^B$	0	0	4	5·5	7	5·5
$\beta^B\beta^B \times \beta^B\beta^B$	No matings					

It is evident that the different phenotypes in these animals can be distinguished by paper electrophoresis on the basis of β-globulin components of different mobilities. In the simple phenotype system observed in goats (see later), paper electrophoresis was in fact used successfully for independent typing of the sera. From an observation of Smithies[3], it seems likely that human phenotypes could be distinguished on paper also.

Consideration of the electropherograms of homozygous sera shows that each β-globulin zone on paper corresponds to a group of zones in starch-gel electrophoresis, the number of zones in the group depending on the species. In sheep and goats there is a faint zone followed by an intensely staining zone, whereas in cattle there is a faint zone followed by a moderately staining zone and two intensely staining zones, described in order of decreasing mobility in the gel.

Comparison of the electropherograms of homozygous and heterozygous sera shows that some of the zones into which the β-globulin components are resolved in starch gel are superimposed in some of the heterozygous sera, particularly in cattle. When this occurs, resolution by paper electrophoresis becomes incomplete and the β-globulins usually appear as a broad zone. This superimposition also makes it difficult to apply a system of nomenclature based on individual coding of the starch gel zones[5] without knowing more about the resolving mechanism and interrelations of the zones in starch gel[6].

β-Globulin polymorphism of goats. Sera from about sixty goats of the British Saanan breed at the Rowett Research Institute have been examined by starch gel electrophoresis using phosphate buffer[1] and by paper electrophoresis. This revealed three phenotypes shown in the electropherograms of Fig. 3. (The two methods are compared above.)

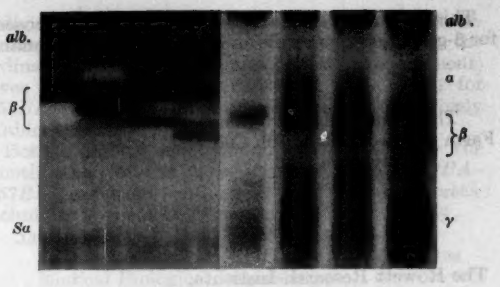

Fig. 1. Electropherograms of sheep sera showing β-globulins of sheep sera of four different phenotypes. Left, starch gel; right, paper electrophoresis. Key: α, β and γ = α-, β- and γ-globulins. *alb.*, albumin; *Sα*, slow alpha zone

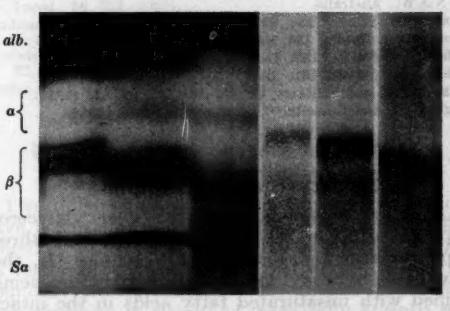

Fig. 2. Electropherograms showing β-globulins of three homozygous cattle sera. Left, starch gel; right, paper electrophoresis. Key: as Fig. 1

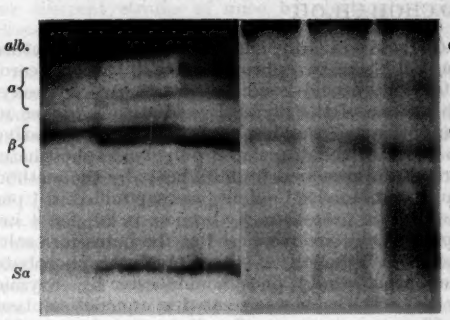

Fig. 3. Electropherograms of goat β-globulin phenotypes. Left, starch gel; right, paper electrophoresis. In each case the phenotypes are from left to right $\beta^A\beta^A$, $\beta^A\beta^B$ and $\beta^B\beta^B$. Key: as Fig. 1

Some animals were examined twice with intervals of up to a year and the phenotype found to be constant. It was also found that mixed sera of the two outermost phenotypes shown in Fig. 3 were indistinguishable from the third (centre) when run together in starch gel. This suggested that the β-globulins of goats are controlled by two allelomorphs β^A and β^B, giving rise to the genotypes β^A/β^A, β^A/β^B and β^B/β^B, and the three phenotypes $\beta^A\beta^A$, $\beta^A\beta^B$ and $\beta^B\beta^B$. This hypothesis is supported by mating results shown in Table 1, where the observed and expected distribution of phenotypes of some forty offspring are compared.

This genetic mechanism is similar to that proposed for β-globulin polymorphism in cattle, sheep and man.

G. C. Ashton*

The Animal Health Trust
Farm Livestock Research Centre,
Lilystone Hall,
Stock,
Essex.

E. I. McDougall

The Rowett Research Institute,
Bucksburn,
Aberdeenshire.
July 3.

* Present address: Sheep Biology Station, P.O. Box 144, Parramata, N.S.W., Australia.

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Structure of Cardiolipin

Pangborn[1] considered that the glycerylglycerophosphate skeleton of cardiolipin contained three phosphoric acid and four glycerol residues (I), the six available hydroxyl groups in this structure being esterified with unsaturated fatty acids in the intact phospholipid.

(I) $CH_2OH.CHOH.CH_2O.P : O(OH).CH_2O.CHOH.CH_2O.P : O(OH).CH_2O.CHOH.CH_2O.P : O(OH).CH_2O.CHOH.CH_2OH$

Macfarlane and Gray[2] suggested a simpler structure for cardiolipin of two phosphoric acid, three glycerol and four fatty acid residues, with a free hydroxyl group on one of the glycerol residues. Evidence in favour of this structure has been obtained by oxidative degradation of the deacylated polyglycerophosphate.

Cardiolipin, prepared from ox heart by the method of Faure and Morelec-Coulon[3], was saponified in 1 per cent ethanolic potassium hydroxide at 20° for 4 hr. The phosphorus was recovered as the potassium salt, insoluble in ethanol, of the polyglycerophosphate (orthophosphate nil; phosphomonoester by enzymic assay nil). The ester was treated in aqueous solution with 50 per cent excess of sodium periodate at 20° for 30 min., and the formaldehyde produced was estimated colorimetrically with chromotropic acid; found, molar ratio formaldehyde : phosphorus, 1·03 : 1·0. The iodates were removed as the insoluble barium salts and the formaldehyde by repeated evaporation of the solution to dryness in a vacuum desiccator. The phosphorus was recovered as the soluble barium salt of a complex ester (orthophosphate and phosphomonoester, nil). The aldehyde content of the ester was estimated by oxidation with hypoiodite by the method of Macleod and Robison[4]; found, 0·87 mole aldehyde/mole phosphorus. The results indicate that the polyglycerophosphate skeleton of cardiolipin consists of three glycerol residues linked by two phosphoric acid residues (II); on oxidation of the terminal glycerol residues with periodate II would give 2 moles of formaldehyde and glyceroldi-(formylmethyl hydrogen phosphate) (III). The ester linkages are assigned arbitrarily to the α position.

$CH_2OH.CHOH.CH_2O.P : O(OH).CH_2O.CHOH.CH_2O.P : O(OH)CH_2O.CHOH.CH_2OH$ (II) →
$CHO.CH_2O.P : O(OH).CH_2O.CHOH.CH_2O.P : O(OH).CH_2O.CHO$ (III) $+2H.CHO$

Marjorie G. Macfarlane

Lister Institute of Preventive Medicine,
London, S.W.1.
Aug. 5.

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Different Types of Hæmoglobin in Two Strains of Mice

While studying the therapeutic effect of homologous bone marrow transplantation in irradiated mice we needed a simple and sensitive technique to differentiate between the hæmatopoietic activity of the host and that of the graft. The possibility of employing differences between the hæmoglobins of our two strains of mice (*CBA* and *C57BL*) and their hybrid was suggested by the findings of Ranney *et al.*[1,2], who demonstrated two different hæmoglobin patterns in eight inbred strains of mice by using paper electrophoresis.

In the present paper the results are described of two kinds of experiments on hæmoglobin, namely, electrophoresis in agar gel and denaturation with alkali.

Agar gel electrophoresis was carried out as described by Robinson *et al.*[3] with some minor modifications. Glass plates, 82 × 82 mm., were carefully cleaned with chromic acid and provided with a coating by submerging them in 0·3 per cent agar and drying at 60° C.; 9 ml. of a 1 per cent solution of agar in 0·05 *M* citrate buffer *p*H 6·5 was poured on to each and allowed to solidify. Electrical contact between buffer vessels and the agar layer was made with filter paper strips, saturated with the citrate buffer. The hæmoglobin solutions were prepared by lysing the washed erythrocytes of 0·04 ml. of tail blood with 0·05 ml. of glass-distilled water for 15 min. at room temperature. Thereafter 0·05 ml. of citrate buffer was added and the stromata were

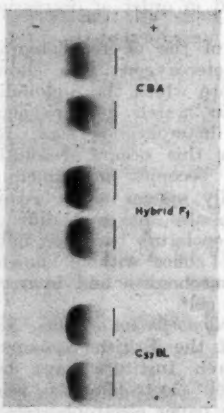

Fig. 1. Electrophoretic pattern of *CBA*, hybrid F_1 and *C57BL* mice

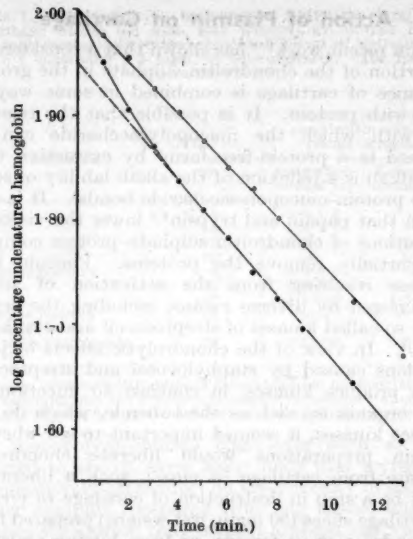

Fig. 2. Alkali denaturation of mouse hæmoglobin with 0·01 *N* sodium hydroxide. O—O—O, *C57BL* (mean of six); ●—●—●, *CBA* (mean of seven)

centrifuged down at 12,000 *g* (Servall centrifuge with the *SS*1-head, 9,600 r.p.m.). Electrophoresis was carried out in a cold chamber at 2° C., usually for 3–5 hours. The runs were made at 80 V., which resulted in a current of 19–21 m.amp. with one plate in our electrophoresis apparatus.

A typical electrophoretic pattern of mouse hæmoglobin is shown in Fig. 1, from which it may be concluded that hæmoglobin of *C57BL* mice has only one component, whereas hæmoglobin of *CBA* has two. The hæmoglobin of the hybrid F_1 (*CBA* ♂ × *C57BL* ♀) does not show a sharp boundary, but we have not been able to distinguish a second component. Recently, Dr. Mathé has informed us that a heterogeneity in the hæmoglobin of six strains of mice could be demonstrated by starch gel electrophoresis.

The alkali-denaturation velocity was measured as described by Jonxis and Visser[4] except that 0·01 *N* sodium hydroxide was used instead of 0·06 *N*, mouse hæmoglobin being much more labile to alkali than human hæmoglobin.

The results obtained with this method agree with those of the electrophoretic technique. On theoretical grounds it is to be expected that, for homogeneous hæmoglobin, the logarithm of the percentage denaturation is a linear function of time, whereas for heterogeneous hæmoglobin the function does not become linear until the more rapidly denatured component is totally converted[5]. This has been found to be the case, as may be seen from Fig. 2, which gives the denaturation curves for *C57BL* (mean of six determinations on different animals) and *CBA* mice (mean of seven). There is, however, a considerable variation in the slope of the curves for individual mice of the same strain, but the intersection of the linear part of the curves with the ordinates (percentage of slowly denatured component) gives significantly different values ($P < 0.01$) for *C57BL*, *CBA* and hybrid F_1 mice respectively (Table 1).

Table 1. Percentage of Slowly Denatured Component of *C57BL*-, *CBA*- and Hybrid F_1 Mouse Hæmoglobin

Type of hæmoglobin	No. of experiments	Percentage of slowly denatured component ± *S.D.*
C57BL	6	100 ± 0·5
CBA	7	88 ± 0·7
Hybrid F_1	6	94 ± 0·8

Both methods have proved to be useful for the identification of the type of hæmoglobin in *CBA*–*C57BL* radiation chimæræ. The electrophoretic technique is preferable, because of its simplicity.

W. Welling
D. W. van Bekkum
Medical Biological Laboratory,
National Defence Research Council T.N.O.,
Rijswijk-Z.H., Netherlands. July 7.

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Different Heterogeneities of Mouse Hæmoglobin according to Strains

Two different electrophoretic hæmoglobin patterns have been demonstrated by paper electrophoresis in inbred strains of mice, and these have been shown to be under genetic control[1,2]. By means of moving-boundary electrophoresis, four different peaks have been observed in strains of genetically anæmic mice (ref. 3 and Cook, J. (personal communication) has observed three major peaks and two minor ones by chromatography on 'Amberlite *X*64'). In studies on two different strains of mice by electrophoresis in gelosis, Van Bekkum, D. W., and Welling, W. (personal communication), have demonstrated in one of them the existence of a single hæmoglobin component, and in the other the existence of several components. This communication describes the different patterns obtained by starch gel electrophoresis of hæmoglobins from six inbred strains of mice.

The strains were as follows: *C57Bl*6, *C57Br*, *AKR*, Swiss, *DBA*2 and *C3H*. Blood samples were drawn from several mice in each strain by puncture of the cavernous sinus. A 10 per cent hæmoglobin solution was prepared for immediate use by Drabkin's method[4] and stored at 4° C. Electrophoretic runs were performed within 24 hr. of the venous puncture, since the spontaneous denaturation of mouse hæmoglobin seems to be much faster than that of human hæmoglobin. Starch gels were prepared according to Smithies[5] and electrophoresis performed as described by de Grouchy *et al.*[6] (*p*H, 8·5; 6 V./cm.; 5 hr.).

Table 1. The Four Types of Hæmoglobin Pattern observed in Six Inbred Strains of Mice

	Description of pattern	Name of strains
Type 1 (Fig. 1*a*)	One very important major band. Two fringes, the more slowly moving one being slightly more important	*C57Bl*6
Type 2 (Fig. 1*b*)	Two major bands	*AKR*
	One minor slowly moving band	*C3H*
Type 3 (Fig. 1*c*)	Three major fast moving bands	
	One minor slowly moving band	*C57Br*
	A certain number of *C57Br* did not exhibit one of the two slowly moving bands, thus, two sub-groups in type 3 may exist	
Type 4 (Fig. 1*d*)	Three major bands	Swiss
	Two minor bands	*DBA*2

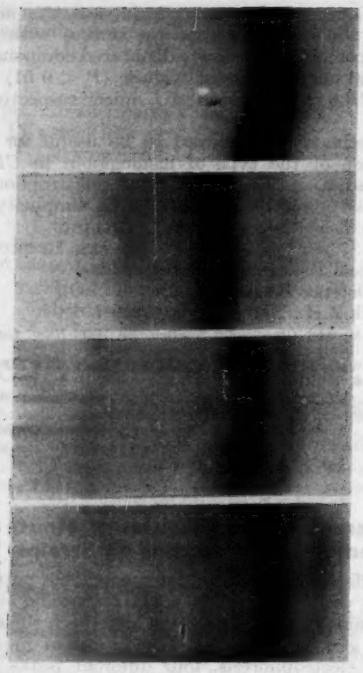

Fig. 1. Starch gel electrophoresis of mouse hæmoglobin. Four different patterns corresponding to different inbred strains

Electrophoretic patterns are characterized by a number of discrete bands which vary from one strain to another. The largest number of bands observed is five. It is thus possible to classify the seven strains of mice studied into four different groups according to their hæmoglobin pattern. Fig. 1 shows a typical pattern of each type and Table 1 is a summary of our results.

The high resolving power of starch gel electrophoresis permits the identification in mice of a larger number of hæmoglobin fractions than does paper electrophoresis or moving-boundary electrophoresis. Furthermore, it is possible to distinguish four different groups of inbred strains of mice, while only two were known previously.

J. Rosa
G. Schapira
J. C. Dreyfus

Laboratoire de Biochimie,
Hôpital des Enfants Malades,

J. de Grouchy

Clinique de Génétique Médicale,
Hôpital des Enfants Malades,

G. Mathé
J. Bernard

Centre de Recherches de
l'Association Claude Bernard,
Paris. July 7.

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Action of Plasmin on Cartilage

Much recent work[1-4] has shown that a considerable proportion of the chondroitin sulphate in the ground substance of cartilage is combined in some way or other with protein. It is possible that the relative ease with which the mucopolysaccharide can be obtained in a protein-free form[5] by extraction with mild alkali is a reflexion of the alkali lability of some of the protein-mucopolysaccharide bonds[4]. It is also known that papain and trypsin[3,4] lower the viscosity of solutions of chondroitin sulphate–protein complex and partially remove the proteins. Plasmin is a protease resulting from the activation of serum plasminogen by diverse means, including the action of the so-called kinases of streptococci[6] and staphylococci[7,8]. In view of the chondrolytic effects of joint infections caused by staphylococci and streptococci which produce kinases, in contrast to infection by other organisms, such as the tubercle, which do not produce kinases, it seemed important to see whether plasmin preparations would liberate chondroitin sulphate from cartilage *in vitro*: such a liberation might be a step in destruction of cartilage *in vivo*.

Cartilage slices (50 mgm. wet weight) prepared from the condyles of ox femora, or from human articular cartilages, were incubated at 35° C. with phosphate–citrate buffer[9] (*p*H 6·2, *M*/10 with respect to phosphate) and chloromycetin (0·1 mgm./ml.) to prevent bacterial contamination, to which was added (*a*) varying amounts of a preparation of bovine plasmin (Parke Davis), (*b*) plasmin + an antiplasmin preparation. These mixtures, together with suitable controls, were incubated at 35° C. on a shaker in screw-capped bottles and at the end of varying times the fluid in the bottles was withdrawn, the cartilage slices washed with buffer and the combined washings and supernatant fluids centrifuged. The relative amount of chondroitin sulphate in the supernatant fluids was estimated approximately by two methods: (*a*) a sample of the supernatant was mixed with buffer at *p*H 4·2 and then acidified serum added under the conditions of Tolksdorff *et al.*[10]; the turbidity obtained was then compared with that given by known amounts of a commercial sample of chondroitin sulphate. That the turbidity obtained with the supernatant fluids was due to chondroitin sulphate was shown by incubating them with either testicular or staphylococcal hyaluronidase at *p*H 6·0: the former hydrolyses chondroitin sulphate, whereas the latter does not. (*b*) By hydrolysing samples of the fluids with 4 *N* hydrochloric acid for 4 hr. at 100°, removing the hydrochloric *in vacuo* and estimating the amount of hexosamine by the method of Elson and Morgan[11]. Table 1 shows the type of results which have been obtained with bovine cartilage. Liberation of chondroitin sulphate from bovine cartilage slices was more complete in the presence of the plasmin preparation and was stopped by so-called antiplasmin. The discrepancy between the amounts of chondroitin sulphate estimated by methods (*a*) and (*b*) presumably reflects either the lack of purity of the chondroitin sulphate standard preparation or the different degree of turbidity given by unit weights of the chondroitin sulphate liberated from the cartilage and the standard preparation. Examination of the effect of papain and trypsin on the cartilage slices showed that both of these enzymes also liberate the mucopolysaccharide. The activity of trypsin may argue against the liberation being due simply to hydrolysis of the protein associated with

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Table 1. Liberation of Chondroitin Sulphate from Bovine Cartilage Slices (50 mgm. wet weight) by Bovine Plasmin. The basal incubation medium consisted of 2·0 ml. of McIlvaine buffer pH 6·2 containing 0·2 mgm. of chloromycetin. The incubation temperature was 35°

Additions	Time of incubation (hr.)	Relative liberation of chondroitin sulphate (range in mgm.)	
		Estimation method (*a*)	Estimation method (*b*)*
None	6	0·8–1·4	—
	24	1·3–1·5	—
	42	0·5–1·5	0·2–0·5
	72	—	0·35
4 units plasmin	6	2·0–2·2	—
	24	2·0–5·2	—
	42	5·1–5·8	1·3–2·2
	72	—	3·82
5 units plasmin + 4 units antiplasmin	24	0·5–0·6	0·0–0·5

* mgm. of chondroitin sulphate calculated from hexosamine estimation assuming that the mucopolysaccharide is composed of equimolar amounts of anhydro galactosamine sulphate and glucuronic acid.

the mucopolysaccharide complexes such as those referred to above, since Muir[4] finds that trypsin has comparatively little effect on the viscosity of solutions of her complex, prepared from pig tissue, although Mathews[2] reported that trypsin lowered the viscosity of solutions of his, prepared from ox tissue.

It thus appears possible that, if sufficient plasminogen is present in the vicinity of cartilage *in vivo*, activation by the kinase produced by staphylococcal and streptococcal infections may be sufficient to cause considerable leakage of chondroitin sulphate from the ground substances, with consequent damage to the mechanical properties of the cartilage.

C. H. Lack

Institute of Orthopaedics,
Royal National Orthopaedic Hospital,
Stanmore, Middlesex.

H. J. Rogers

National Institute for Medical Research,
Mill Hill, London, N.W..7

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Experimental Suppression of Increased Capillary Permeability in Thermal Burns in Rats

The possibility has been widely considered that œdema in thermal injury is partly due to release of substances or activation of endogenous mechanisms which increase capillary permeability. There is little convincing evidence in support of such a hypothesis, and the œdema associated with burning has never been suppressed by pharmacological means[1].

Using a modification of the method described by Sevitt[2] to produce standard burns, we have been able to confirm his observations on the development of both an immediate and a delayed œdema by subjecting rats to a standard burn (55° C. for 27 sec.) on their abdomen.

Increased capillary permeability was estimated by leakage of protein-bound trypan blue into the superficial layers of the burnt area (estimated visually on a unitary scale from 0–10) and by measuring the water content of a standard area of skin and subcutaneous tissue in the burnt zone.

In control rats, dye-accumulation in the superficial layers began about 10–20 min. after injury and reached a maximal intensity about 60–90 min. later. The accumulation of œdema in the deeper tissues developed at a slow rate and was maximal 2–3 hr. after burning (Table 1).

In rats pretreated with mepyramine maleate ('Anthisan') (1 mgm./kgm.)[3] there was a significant delay in the accumulation of dye in the superficial layers of the burnt area (Table 1). By 60–90 min., however, no difference between the control and treated animals was discernible. There was a similar, though less striking, delay in the development of subcutaneous œdema. This was apparent only in estimations made up to 1 hr. after burning. At 3 hr. after injury there was no significant difference between the œdema of the deeper tissues of the control and treated groups.

In other rats pretreated with sodium salicylate[4] (0·66 gm./kgm. body-weight given intraperitoneally 30 min. before injury), there was a striking suppression of both dye-accumulation and œdema formation, neither of which ever reached the values found in control animals.

A third group of rats was given 3 mgm./kgm. body-weight of di-*iso*propylfluorophosphonate in arachis oil intramuscularly, 30 min. before burning. Up to 30 min. after injury there was an inhibition of dye-leakage similar to that observed in rats given mepyramine. Thereafter, however, staining of the burnt area developed more slowly than in the group treated with mepyramine and in many cases never reached the intensity observed in untreated animals. Leakage of protein-bound dye was nevertheless more extensive 3 hr. after burning in the di-*iso*propylfluorophosphonate group than in the salicylate group. However, rats treated with di-*iso*propylfluorophosphonate showed a reduction in the amount of œdema developing in the deeper tissues of the burnt area when measured 3 hr. after injury, as striking as that observed in animals treated with salicylate.

Table 1. Effect of Various Measures on the Accumulation of Trypan Blue and of Œdema Fluid in the Skin of Rats subjected to Thermal Burns

Treatment	No. of animals	Time after burn	Water* (gm./cm.² burnt skin)	Grading of blue in arbitrary units
Controls	17	30 min.	—	4·6±1·7
	20	3 hr.	0·15±0·03	9·5±1·03
'Anthisan'	11	30 min.	—	1·7±1·28 ($P = < 0·001$)
	10	3 hr.	0·15±0·059 (N.S.)	9·5±1·06 (N.S.)
Salicylate	12	30 min.	—	0 ($P = 0$)
	8	3 hr.	0·05±0·02 ($P = < 0·001$)	2·4±0·92 ($P = < 0·001$)
Di-*iso*propyl-fluorophos-phonate	8	30 min.	—	1·6±0·9 ($P = < 0·001$)
	9	3 hr.	0·07±0·04 ($P = < 0·001$)	5·0±1·5 ($P = < 0·001$)

* Mean water content of normal skin = 0·03 gm./cm.².
—, not tested.
N.S., not significant.

Depletion of bodily histamine and 5-hydroxytryptamine by a course of injections of compound 48/80 also delayed the onset of dye-linkage into the burnt area. BOL 148, an antagonist of 5-hydroxytryptamine, caused only a slight and inconstant delay in the onset of dye-leakage.

These results, which will be published in greater detail elsewhere, are consistent with the hypothesis that delayed increased capillary permeability seen after thermal injury is due to the consecutive operation of two or more intermediary mechanisms or chemical mediators released from the tissues. They also indicate that release of histamine may be responsible for the initial phase of such a series of events. Similar results have been obtained in inflammation due to chemical irritation[3,4]. The action of salicylate and di-*iso*propylfluorophosphonate on the later stages of the reaction to burning may be due to inhibition of the activation of globulins which increase capillary permeability. However, di-*iso*propylfluorophosphonate in particular has general effects on capillary permeability which are of interest and are the subject of further investigation.

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W. G. Spector
D. A. Willoughby
Department of Morbid Anatomy and Medical Unit,
University College Hospital Medical School,
London, W.C.1. Aug. 23.

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Straight-Growth Inhibitors in Glucose

Several authors[1–5], when partitioning crude ether extracts of plant material, have saturated the aqueous phase with glucose to facilitate its separation from the ether phase.

During the course of an investigation on growth correlation inhibitors, it was found several times that extracts prepared with the addition of glucose inhibited straight growth of wheat coleoptile sections more than those without glucose. After checking other sources of inhibitory substances, it was decided to investigate the effect of glucose more fully.

Saturated solutions of glucose (usually 45 ml.) were made alkaline with an 8 per cent w/v solution of sodium bicarbonate (1–9 ml. of solution) and were shaken three times with 30 ml. of peroxide-free ether. The ether phase was discarded and the aqueous solutions acidified to *p*H 3·1 with orthophosphoric acid, and again shaken three times with 30 ml. ether. The three ether fractions were combined and reduced to 1 ml. on a water bath. Amounts equivalent to 10 gm. glucose were delivered on to Whatman No. 1 paper strips with a microsyringe[6]. Comparisons were made with extracts from 7–8 days old etiolated pea seedlings grown at 25° C. (the top inch of the stem was discarded to avoid a possible effect of indoleacetic acid on the inhibitor assay). For every 10 gm. of pea stems 1 ml. of distilled water was added to the crude ether extract, and ether evaporated and the aqueous solution filtered (Whatman No. 1). This aqueous extract was treated in the same way as the extract from the glucose solution, except that the amount of ether extract applied per chromatogram was equivalent to 20 gm. fresh weight. Chromatograms were developed in the ascending direction after an equilibration period of 6–12 hr. with the solvents given in Fig. 1. Solvents containing acetic acid were unsatisfactory, for they made the coleoptile sections flaccid and caused inhibition. This effect could not be overcome without affecting the natural inhibitors as well. Chromatograms were cut into ten equal strips, which were tested against two control strips from the same chromatogram. Nitsch's assay technique[7] was used with the minor modifications that 10-mm. sections were cut from wheat coleoptiles of the variety Cross 7.

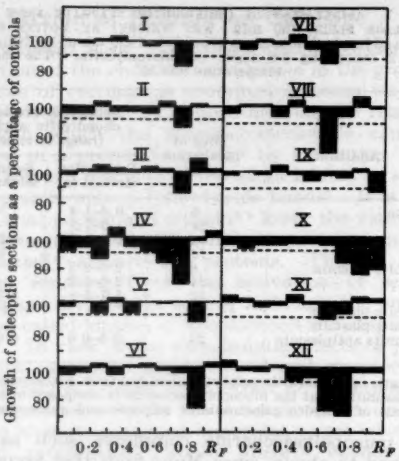

Fig. 1. The response of wheat coleoptiles to 10 gm. of different samples of glucose (I, A.R. grade; II and III, samples obtained from H. F. Stevens, Christchurch, in 1953 and 1958 respectively), and 20 gm. of stems of etiolated pea seedlings (IV); solvent, butanol/25 per cent ammonia/water (100 : 8 : 100). V–XII, glucose and peas using different solvents: V (glucose) and VI (peas), 96 per cent ethanol/25 per cent ammonia (9 : 1); VII (glucose) and VIII (peas), *iso*propanol/25 per cent ammonia/water (8 : 1 : 1); IX (glucose) and X (peas), 70 per cent ethanol; XI (glucose and XII (peas), distilled water. Growth has been plotted as a percentage of the growth of control sections; the broken line represents the level at which differences are significant ($P = 0{\cdot}05$). The mean growth of control sections in different experiments was 7·88–8·84 mm. over a period of 17–18 hr.

Results are illustrated in Fig. 1. They show that all three samples of glucose of different origin (one of which was A.R. grade) contained a straight-growth inhibitor the position of which on the chromatograms was identical with that of 'inhibitor β' which occurs in etiolated pea seedlings (I–IV). The same held true for four other solvent systems (V–XII).

In another experiment the inhibitors from 250 gm. of glucose and from 250 gm. of peas were eluted from several chromatograms, and the concentrated eluates were applied to paper strips in a wide range of con-

Table 1. The Effect of the Inhibitors in Peas and in Glucose eluted from Chromatograms, at Various Concentrations

Concentration*	Growth of coleoptile sections as a percentage of controls†	
	Pea stems	Glucose
100 *G*	54·7	72·3
10 *G*	83·2	90·0
1 *G*	88·7	91·8
0·1 *G*	101·3	91·8
0·01 *G*	94·3	97·9

* *G* is equivalent to 1 gm. fresh weight of pea stem tissue or 1 gm. of glucose respectively.
† Significant differences ($P = 0{\cdot}05$) from controls 10·8 and 9·5 per cent respectively.

centrations. Again, the results (Table 1) show a similar pattern for both inhibitors, although on a dry weight basis the amount of inhibition was much less in glucose than in peas.

Varga and Ferenczy[8] detected 'inhibitor β' in fruits of thirty different species belonging to ten families and the many references listed by Ferenczy[9] illustrate its widespread occurrence. This report, the fact that glucose is prepared from plant sources, and the identical position on chromatograms prepared with five different solvents suggest a possible similarity between the inhibitor found in peas, and glucose. Apart from this suggestion, this communication is intended as a warning against the use of inadequately purified glucose when partitioning extracts which are to be assayed for growth substances by the coleoptile straight growth test.

R. F. M. Van Steveninck
Crop Research Division,
Department of Scientific and Industrial Research,
Christchurch, New Zealand.

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Use of Lyophilized Formol-treated Red Cells in Blood-Group Serology

Moskowitz and Carb[1] have reported that treatment of human red cells with formalin inhibits their agglutinability without impairing their absorptive power for the corresponding blood-group agglutinin. During the course of a similar investigation, we have confirmed these findings and also the observation of Weinbach[2] that formol-treated red cells are sufficiently 'toughened' to withstand freeze-drying by lyophilization. We have found that such lyophilized cells re-suspended in normal saline retain their original absorptive capacity for blood-group agglutinins, and that eluates prepared from these cells by Landsteiner's heat method, following absorption, are as active as those from untreated cells. No hæmolysis has occurred during elution.

So far as we are aware, lyophilized formol-treated red cells have not previously been put to practical use in blood-group serology, and we wish therefore to direct attention to a practical application of this technique. We encountered recently a sample of red cells which reacted similarly to certain rare cells described by Weiner *et al.*[3] and given the symbol A_m, which showed a marked hæmolysis when we attempted to store them at low temperature in glycerol. These red cells were not agglutinated by group O immune type anti-A sera but readily absorbed anti-A. Exposure to formol-saline and lyophilization enabled these cells to be preserved satisfactorily without impairing their power of absorption and subsequent elution.

The technique we have used in the initial treatment with formalin differs in some respects from that of Moskowitz and Carb and is as follows. One volume of formaldehyde solution (analytical reagent, approximately 36 per cent w/v HCHO) diluted 1 in 30 with non-buffered normal saline is incubated at 37° C. with an equal volume of washed packed red cells. After 12–24 hr. incubation the treated red cells are washed three times in normal saline and then dried from the frozen state. If immediate lyophilization is not convenient, the formol-treated cells may be stored under distilled water at 4–6° C. Storage under distilled water has proved more satisfactory than under normal saline since, while some degree of hæmolysis tends to occur with the latter, formol-treated red cells stored for up to one week under distilled water have shown no trace of hæmolysis.

E. R. Gold
W. J. Lockyer
Geoffrey H. Tovey
National Blood Transfusion Service,
Southmead, Bristol. July 28.

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Relationship between Certain Biological Constants and Trophic Condition in Mosquito Populations

Macdonald[1] has shown that the composition of mosquito populations, as regards the proportion in the parous state, can be calculated theoretically, yielding an equation which involves the daily survival-rate and a time period based on the gonotrophic cycle. This, and a modified equation given by Davidson[2], have provided a useful means for calculating daily survival rates in the field. Using similar methods, it is possible to derive a general expression which determines the composition of populations as regards the proportion in the unfed condition.

Assuming a population in a steady equilibrium, with a daily hatch of M mosquitoes, and a daily survival-rate of p, then the number aged x days will be Mp^x. Then let: n = days from hatch to first feed; d = days developing eggs, up to the first oviposition; f = days from oviposition to next feed; $c = d + f$.

This cycle will be repeated every c days. Then, numbering the days after emergence 0, 1, 2, . . . etc., the days on which mosquitoes are in a given state are as shown below:

Unfed

0, 1, 2	$n-1$
$n+d,\ n+d+1,\ n+d+2$	$n+c-1$
$n+c+d,\ n+c+d+1,\ n+c+d+2$	$n+2c-1$

Fed and/or developing eggs

$n,\ n+1,\ n+2$	$n+d-1$
$n+c,\ n+c+1,\ n+c+2$	$n+c+d-1$
$n+2c,\ n+2c+1,\ n+2c+2$	$n+2c+d-1$
etc.	

Let G = number which are fed and/or developing eggs;

$$\text{then } G = M(p^n + p^{n+c} + p^{n+2c} \ldots + p^{n+1} + p^{n+c+1} + p^{n+2c+1} \ldots + p^{n+d-1} + p^{n+c+d-1} + p^{n+2c+d-1} \ldots)$$

$$= M\left(\frac{p^n}{1-p^c} + \frac{p^{n+1}}{1-p^c} + \frac{p^{n+2}}{1-p^c} \ldots + \frac{p^{n+d-1}}{1-p^c}\right)$$

$$= \frac{M}{1-p^c}\left[(p^n + p^{n+1} + p^{n+2} \ldots + p^\infty) - (p^{n+d} + p^{n+d+1} + p^{n+d+2} \ldots + p^\infty)\right]$$

$$= \frac{M}{1-p^c}\left(\frac{p^n}{1-p} - \frac{p^{n+d}}{1-p}\right)$$

$$= \frac{M(p^n - p^{n+d})}{(1-p)\ (1-p^c)}.$$

Let P = total population of mosquitoes;

then $P = M(1 + p + p^2 + p^3 \ldots p^x)$

$$= \frac{M}{1-p}$$

Therefore, if g = proportion of the population which is fed and/or developing eggs;

then $g = \frac{G}{P} = \frac{p^n - p^{n+d}}{1-p^c}$

and, if u = proportion in the unfed state,

$$u = 1 - \frac{p^n(1-p^d)}{1-p^c}$$

Two special cases may be noted, which yield simplified equations:

(*a*) where $n = f$; then $u = \frac{1-p^f}{1-p^c}$;

(*b*) where $f = 0$; that is, where oviposition and refeeding occur on the same night;

$$\text{then } u = 1-p^n$$

Thus, if the proportions u or g are known from representative samples of mosquitoes from natural resting places, a knowledge of any three of the quantities p, n, d and c allows calculation of the fourth. In practice, this will usually mean calculation of survival rate from details of the gonotrophic cycle. The method has been tested with four Malayan species of *Culex*, the survival rates of which have been estimated by other means, and close agreement has been found; these results will be published elsewhere. This suggests that, in certain species at least, the above relationship may provide a useful means for following alterations in survival rate, for example, during control campaigns, although where anomalous gonotrophic cycles occur, for example, with a pregravid phase, the equations will require modification.

Donald H. Colless

Department of Parasitology,
University of Malaya.
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Effect of Nitric Oxide on the Radiosensitivity of Tumour Cells

Following the important observation of Howard-Flanders[1] that the presence of a low concentration of nitric oxide in the medium in which bacteria are suspended at the time of irradiation confers a level of radiosensitivity comparable with that of an equal concentration of dissolved oxygen, we have investigated the effects of nitric oxide on the radiosensitivity of tumour cells.

As judged by the time of appearance of tumours which arise from a standard inoculum of irradiated cells, we find that nitric oxide is capable of raising the level of radiosensitivity of anoxic tumour cells to that which they would have if irradiated in the presence of dissolved oxygen.

The Ehrlich ascites tumour cells used for irradiation were harvested from white mice (Fox) which had been inoculated five days previously with $\sim 2 \times 10^7$ cells. Since nitric oxide readily combines with hæmoglobin, erythrocytes were removed as completely as possible by two osmotic shock treatments in distilled water, terminated after 40 sec. by addition of double-strength saline. In this, and in most other respects, our technique followed closely that which had been elaborated by Scott for the study of factors which may influence the radiosensitivity of tumour cells[1]. Cells were finally suspended in saline for irradiation at 5×10^7 cells/ml. The nitric oxide was prepared by adding glacial acetic acid to a warm concentrated solution containing sodium nitrite and ferrous sulphate. After washing the gas to remove traces of nitrogen dioxide, oxygen was excluded as rigorously as possible at all stages. All cell suspensions were rendered strictly anaerobic by gas exchange with an atmosphere of oxygen-free nitrogen (British Oxygen Co.) and by their own respiration in this atmosphere before they were irradiated or treated with nitric oxide. A 4-ml. sample of the cell suspension containing 2×10^8 cells was exposed to 1,200 r. of 200 kV. X-rays filtered through 0·5 mm. copper and 1 mm. aluminium at 325 r./min. in a 'Perspex' vessel through which gas could flow (Deschner, E. E., and Gray, L. H., in preparation). In two experiments the nitric oxide was added to the saline cell suspension in the form of a solution in $1{\cdot}5\ 10^{-3}$ M ferrous sulphate, and a slow stream of nitrogen passed through the vessel during irradiation as a safeguard against the entry of oxygen. At the end of each irradiation or control treatment the nitric oxide was displaced by a vigorous stream of nitrogen before the cells were exposed to air. Finally, 0·2 ml. inocula from the treated fluid were introduced into each of six mice. In these experiments a control was run which showed that the effect of the mixture in raising the sensitivity of the cells was attributable mainly to the nitric oxide and not to the ferrous sulphate. In the remaining experiments the ferrous sulphate was dispensed with. Cell suspensions were brought into equilibrium with a gas phase which contained 100, 20, 10 or 2 per cent nitric oxide/nitrogen mixtures, and the same gas was passed over the cells during irradiation.

In most experiments the cells were in the presence of nitric oxide for a maximum of 15 min. Fig. 1 shows that tumours appear in animals inoculated with cells which had been treated with nitric oxide as soon as in animals which received control inocula. Contrasting this with the larger difference in the time of appearance of tumours which arose from cells irradiated in nitrogen and in oxygen, it is evident that any toxicity the nitric oxide may have had was quite insignificant as regards ultimate cell survival. Likewise, exposure of cells to 1,200 r. under completely anoxic conditions, and in the absence of nitric oxide, only slightly delayed the appearance of tumours. When, however, the cells were irradiated in the presence of nitric oxide the appearance of tumours was much delayed. In some cases, no tumours appeared at all within the 30-day observation period, and in every one of 18 irradiations in which nitric oxide was added before irradiation at an initial concentration greater than 10 μM the appearance of the tumours was delayed compared with that of tumours which grew from cells which had been treated with nitric oxide only or exposed anaerobically to X-rays. This evidence, however, is not in itself sufficient to establish the radiosensitizing action of the nitric oxide, since it is possible, in principle, that there may be synergism between the two separate

TABLE 1. EFFECT OF NITRIC OXIDE ON RADIATION DAMAGE TO ASCITES TUMOUR CELLS

		Number of mice showing ascites tumours 10 days after inoculation of 10^7 cells						
		Ferrous sulphate solution		Simple solution			Total	Per cent
Nitric oxide concentration (when used) / Nitric oxide in gas phase		10^{-3} M*	10^{-3} M*	2×10^{-3} M or 4×10^{-4} M, 100 or 20 per cent NO/N_2	4×10^{-4} or 2×10^{-4} M, 20 or 10 per cent NO/N_2	4×10^{-5} M, 2 per cent NO/N_2		
Without nitric oxide	1. Control	5/5	6/6	6/6	5/5	6/6	28/28	100
	2. 1,200 r. in nitrogen	3/5	4/5	6/6	6/6	6/6	25/28	89
	3. 1,200 r. in oxygen		0/6			0/6	0/12	0
With nitric oxide	4. Nitric oxide only	6/6	5/5	6/6	6/6		23/23	100
	5. Nitric oxide before and during irradiation	2/10	0/3 1/3 6/6 6/6 0/6 0/5	3/5 0/6 5/5	2/6 0/4 0/6 0/6 0/4	0/6 2/6 6/6	33/99	33
	6. Nitric oxide after irradiation	6/6	4/5 4/6 3/6 4/6 4/6 3/3	6/6 6/6 4/6	6/6 6/6 6/6 5/6 5/6	6/6 6/6 4/6	88/104	85

* Total nitric oxide present in solution or complexed with ferrous sulphate.

injuries, namely, that due to the radiation and that due to the chemical toxicity of the nitric oxide, which could be mistaken for radiosensitization. To decide critically between these two alternatives, we concentrated most of our effort on pairs of treatments, in one of which nitric oxide was present before and during irradiation and the 'control' in which the cells were irradiated anoxically but exposed to the same nitric oxide concentration for an equal total time shortly after irradiation. Fig. 1 presents results obtained in one group of experiments in which nitric oxide was used as 20 and 10 per cent nitric oxide/nitrogen mixtures. For comparison, one group is included from another experiment to show the effect of 1,200 r. on the growth of cells irradiated aerobically.

It is quite evident that in all ten groups of animals tested in pairs, nitric oxide given before and during irradiation (broken lines) is more effective than when given after irradiation (full lines). In 4 out of 5 critical pairs, the growth of tumour cells irradiated in the presence of nitric oxide corresponded to a degree of damage as great as that which would have been sustained by cells which were aerobic at the time of irradiation. All the results obtained so far in one pilot and four principal experiments are recorded in Table 1. The figures indicate the status of mice, whether with or without tumours, on the tenth day after inoculation of 10^7 treated cells. We believe that these observations establish that nitric oxide is comparable in effectiveness with oxygen in raising the radiosensitivity of tumour cells with respect to the loss of reproductive integrity of those cells on exposure to X-radiation.

Kihlman[3] has recently reported that nitric oxide has a similar action with respect to the induction of chromosome structural damage in *Vicia* root meristem cells.

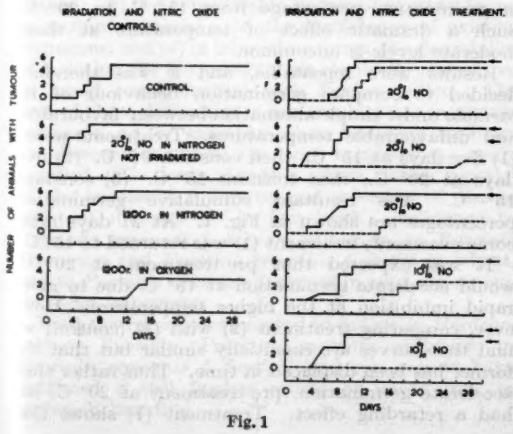

Fig. 1

The fact that there are now three known types of cell, namely, bacteria, plant meristem and mammalian tumour cells in which the molecule of nitric oxide can apparently take the place of oxygen in enhancing sensitivity to X-rays opens new means of exploring the role of oxygen itself.

We are grateful to Dr. O. C. A. Scott, who was prevented by illness from participating in these experiments, for guidance in the procedure for assaying cell survival, and to Dr. D. L. Dewey for assistance in one experiment. One of us (F. O. G.) wishes to acknowledge financial aid during the time of the investigation in the form of fellowship grants from the following groups or sources: the Jane Coffin Childs Memorial Fund for Medical Research; the Osborne Memorial Fund; the Alumni Faculty Research Fund; and the Chemistry Department of Wheaton College.

L. H. GRAY
F. O. GREEN
C. A. HAWES

British Empire Cancer Campaign
Research Unit in Radiobiology,
Mount Vernon Hospital,
Northwood, Middlesex.

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Effect of Maize on Fat Hardness and Growth-Rate in Bacon Pigs

EIGHTEEN Large White pigs (13 females and 5 males) were fed from 100 lb. to slaughter-weight (200 lb.) on rations containing 15, 30, 45, 60, 75 and 90 per cent maize, the rest of the rations consisting of pollards, barley meal, carcass meal and minerals. The crude protein content of the rations varied from 14 to 16 per cent and the three pigs in each group were chosen at random. Feed consumption and live-weight increase were measured, and samples of fat from shoulder, back and loin were taken 24 hr. after slaughter for analysis by the Hanus method for iodine value.

The iodine values of the fats from male pigs were corrected by adding 1·7, as suggested by Lush *et al.*[1], to make them comparable with those from females. They showed wide variation within groups, and the three regression equations for per cent maize on iodine values of shoulder, back and loin fats were significant only at the 20/1 level, with low accountable variance. Nevertheless there was a definite trend in all three sets for fat hardness to decrease as the

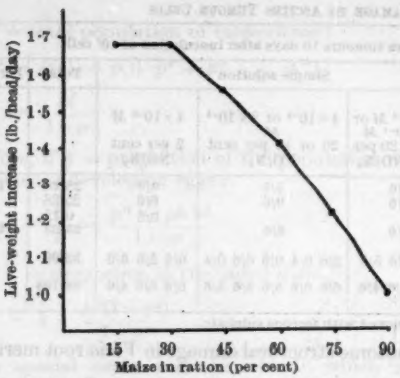

Fig. 1. Effect of maize on growth-rate from 100 lb. to 200 lb. live-weight

percentage of maize in the ration increased, although all the fats were sufficiently hard for curing into bacon and ham. In the factory the fats of 15 pigs were classed as 'very firm', and those of three 'firm', the latter being pigs on 15, 45 and 60 per cent maize.

The percentage of maize in the ration had no significant effect on the feed conversion ratio. There was a wide individual range, from 3·0 to 5·0, while group averages varied irregularly between 3·7 and 4·2. The most efficient individual conversion ratio (3·0) was obtained from a pig on 90 per cent maize, while the least efficient (5·0) was from a pig on 45 per cent maize.

There was a very striking relation between the percentage of maize in the ration and the rate of live-weight increase, and the group averages are plotted in Fig. 1, which shows a regular decrease in daily live-weight gain with increasing proportions of maize above 30 per cent. The regression equation for this relation was significant to more than 1,000/1, with 61 per cent accountable variation. This effect of maize in reducing the rate of growth probably accounts for the tendency for the fat to be softer in pigs on the high-maize rations, since Callow *et al.*[2] found that fast-growing pigs tend to have fat with a low iodine value and slowly growing pigs to have fat with a high iodine value.

The high-maize rations did not appear to lack palatability, and thus the regular decrease in the rate of live-weight gain with increasing percentage of maize in the ration suggests a nutritional deficiency in maize. Krehl *et al.*[3] concluded that zein has an unbalanced amino-acid composition which results in greatly reduced synthesis of nicotinic acid in the body and a higher requirement of tryptophan or nicotinic acid. The tryptophan contents of these rations, calculated from data by Harvey[4] and Fowden and Wolfe[5], were approximately the same, but the proportion of the total tryptophan which was derived from maize increased from 11 to 87 per cent. Nicotinic acid-deficiency in maize was reported by Braude *et al.*[6], who found that young pigs on a diet deficient in nicotinic acid and containing 79 per cent maize failed to gain weight, while another group on the same ration supplemented with 6 mgm. nicotinic acid per head per day gained an average of 5 lb. per week. They concluded that almost all the nicotinic acid in maize is in an alkali-labile 'bound' form which is not available to the pig.

From these results we suggest that the tendency for maize to produce soft fat in bacon pigs may be due to slow growth caused by a higher requirement of tryptophan or a deficiency of 'free' nicotinic acid, and that high-maize rations for pigs may require supplementary nicotinic acid. Other amino-acids may also be involved, and experiments to test these points are now in progress.

A full account of this work will be published in the *East African Agricultural Journal*.

D. W. Duthie
A. F. Dent
H. P. Ledger

East African Agriculture and Forestry Research Organization,
Kikuyu, Kenya.
July 24.

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Temperature and Germination in *Kennedya* Vent.

During the course of studies in the genus *Kennedya*[1] it was considered desirable to determine the germination response of a range of species and ecotypes to different constant-temperature levels. Germinations were made in sand maintained at 60 per cent of the water-holding capacity. A seed was recorded as germinated when the radicle had attained a length of 5 mm. The temperatures used and some typical results are shown in Table 1.

Table 1. Total Germination Percentages after 33 days at Four Temperature Levels

	Species	Line No.	10° C.	15° C.	20° C.	25° C.
1	*K. coccinea* Vent.	*G4*	93·3	94·2	80·8	46·7
2	*K. eximia* Lindl.	*E12*	61·7	72·5	55·8	25·0
3	*K. carinata* Benth. (Domin.)	*X–P6*	90·0	95·8	13·3	3·3
4	*K. prostrata* R.Br.	*A79*	78·3	89·2	5·0	0·8

Optimum temperature (that giving highest percentage at fastest rate) was generally found to be between 15° C. and 20° C. with a sharp decline at 25° C. *Kennedya carinata X–P6* and *Kennedya prostrata A79*, however, showed spectacular decreases in germination percentage from 15° C. to 20° C. Such a dramatic effect of temperature at these moderate levels is uncommon.

Results were repeatable, and it was therefore decided to compare germination behaviour of *K. carinata* under simple alternation between 'favourable' and 'unfavourable' temperatures. Treatments were: (1) five days at 15° C., then constant 20° C. (2) five days at 20° C., then constant 15° C. (3) constant 15° C. The resultant cumulative germination percentages are shown in Fig. 1. At 21 days after commencement, treatment (1) was returned to 15° C.

It was expected that pre-treatment at 20° C. would accelerate germination at 15° C. due to more rapid imbibition at the higher temperature. However, comparing treatment (2) with (3) (control) we find that curves are essentially similar but that the former has been displaced in time. Thus rather than accelerate germination, pre-treatment at 20° C. has had a retarding effect. Treatment (1) shows that

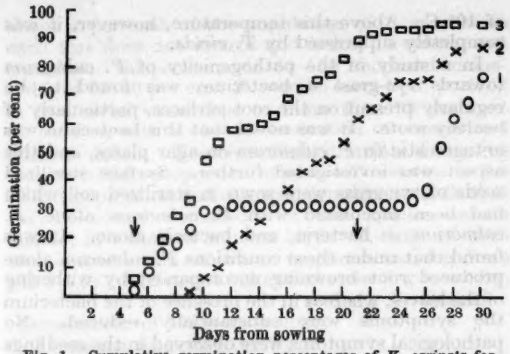

Fig. 1. Cumulative germination percentages of *K. carinata* for (1) 15–20–15° C. (circles), (2) 20–15° C. (crosses), (3) constant 15° C. (squares). Arrows indicate time of transfer

the inhibiting effect of 20° C. is not immediate in action since transfer of seeds from 15° C. to 20° C. did not at once preclude germination: there was a delay of five days before the curve levelled off. This behaviour suggests that 20° C. does not influence all phases of radicle growth but only its commencement. Once initiated in a proportion of the imbibed seeds at the lower temperature, growth continues and results in germination at the higher temperature. No further germination occurs until five days after re-transfer to favourable temperature, thus indicating that the time interval between initiation of radicle growth and completion of germination at 15° C. is approximately five days.

J. H. Silsbury*

Institute of Agriculture,
University of Western Australia,
Nedlands, W.A.
July 24.

*Present address: Waite Agricultural Research Institute, Private Bag No. 1, Adelaide, South Australia.

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Artificial Mutagenesis in Plant Breeding

Many difficulties and disadvantages are involved in undertaking a programme of improvement of crop plants by the artificial induction of mutations. Among those which have been recognized are (*a*) the necessity for screening very large populations of plants; (*b*) the considerable degree of sterility found in mutant material; (*c*) the inefficiency of existing mutagens, and (*d*) in inbreeding forms, the desirability of isolating the frequently male-sterile R_1 generation, to avoid contamination with foreign pollen. The first of these difficulties is less troublesome in certain instances, as for example, when screening seedlings for disease resistance, for large numbers are easily handled. No doubt, for this reason, many reports[1] of the successful induction and selection of disease resistant mutants do in fact exist; resistance to *Erysiphe graminis f. hordeii* in barley, to *Puccinia glumarum* and *Puccinia graminis f. tritici* in wheat, and to *Puccinia graminis f. avenae* in oats, are among the most important.

We have recently attempted to induce in *Hordeum sativum* (variety Proctor) mutants resistant to *Erysiphe graminis f. hordeii*, and our results have indicated a still further source of uncertainty in mutation breeding.

Dormant seeds (water content 10·6 per cent) were exposed to either 10,000 rads of γ-rays from a cobalt-60 source, or to $2{\cdot}2 \times 10^{12}$ thermal neutrons per cm.^{2}, and sown immediately afterwards in field plots. No other variety of barley was grown within a distance of at least one mile in any direction from the plots. R_2 seeds were sown in September, and the seedlings kept under polyethylene cages in a greenhouse. A fortnight after sowing, when the seedlings were at the two-leaf stage, a mixture of conidia, previously collected by suction pump from control plants and stored in talc at 3° C., was blown over the seedlings. Under these conditions there was 100 per cent infection of control plants (reaction type 4, scale 0–4, 4 being completely susceptible) but little sign of infection of a control resistant variety. No attempt was made to isolate the different races of the pathogen, but tests with the differential varieties of Newton and Cherewick indicated that the predominant one present was race 6. 18,490 R_2 seedlings were tested, and the frequency of chlorophyll mutations within them was as follows:

	R_2 plants	Chlorophyll mutants
γ-ray treatment	13,140	104
Thermal neutron treatment	5,350	37

No viable R_2 plant was found showing any degree of resistance to *Erysiphe graminis f. hordeii*.

These results differ strikingly from those of other workers. Hansel and Zakovsky[2], for example, found a similar number of chlorophyll mutations, as well as 190 greenhouse- and 72 field-resistant lines in 15,000 R_2 lines (R_2 ear progenies) of Volkorn barley treated with 9,400 r. X-rays. They attributed this very high rate of induction of resistant mutants to the presence of a large number of loci for this character rather than to a high mutation rate. Knowing the mutation-rate here, then from the mutation-rate of other genes in plants it might be possible to estimate the number of loci involved. If we take only the field-resistant mutants in Hansel and Zakovsky's data, they quote that the values represent two resistant mutants in every 1,000 R_2 seed. Most of the X_2 lines bred true for resistance. 15,000 R_2 ear progenies might represent 5,000 R_2 seed; 10 of these, therefore, will be mutants. Assuming 20 seeds per ear, as they do, and a 3 : 1 segregation of a gene for resistance, the ten R_2 mutant seed would be produced in at least two ears. Therefore, two R_1 ears in 250 had mutant seed (the ear initial is the unit of mutation in a seed). This value assumes no differential intrasomatic elimination of cells containing the mutant gene, and ignores the considerable number of greenhouse-resistant lines. Mutation-rates are known for very few genes in plants. Lewis[3] quotes rates from 0·008 to 0·01 per r. per 10^6 genes for *S* alleles in *Oenothera*, after treatment of pollen mother cells with X-rays, and mutation-rates of the same order have been reported in animals and lower organisms. Though no strictly relevant figures exist, the data do allow us to draw the conclusion that in Volkorn barley there are probably many hundred loci involved in the determination of mildew resistance.

These contradictory results from two separate attempts to induce mildew resistance in barley emphasize yet another difficulty facing a plant breeder attempting to justify a programme of mutation breeding. A thorough knowledge of the

genotype being dealt with, in terms of (*a*) the condition of the gene or genes controlling the character sought, that is, whether dominant or recessive, and (*b*) the number of loci involved, now appears to be of basic importance in making this decision. The fact that similar genes controlling like processes have different mutation-rates[3] is yet another complicating factor. In the absence of such information, and with our present lack of understanding of the process of mutagenesis, and of the factors affecting it, a programme of mutation breeding becomes an extremely speculative affair. The potential value of artificial mutagenesis as a technique remains, however, but a great amount of fundamental work has to be undertaken before its potentiality can be fully exploited.

We are indebted to Prof. K. Mather for guidance in the preparation of this report.

D. Roy Davies
E. T. Wall

Technological Irradiation Group,
Atomic Energy Research Establishment,
Isotope Division,
Wantage Radiation Laboratory,
Grove, Nr. Wantage,
Berks.
Aug. 5.

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Microbial Antagonism of *Fusarium culmorum*

During a survey of soil- and root-invading fungi in swards of perennial rye-grass carried out in 1956 and 1957 particular attention was given to the occurrence of *Fusarium culmorum* (W.G.Sm.) Sacc. This species, which is considered to be a soil inhabitant, is also a weak parasite of the roots of many members of the Gramineae, and during the survey it was isolated regularly from soil and from the roots of rye-grass. We find that its occurrence in both of these substrates is influenced by microbial antagonisms.

In soil, the occurrence of *F. culmorum* showed marked seasonal variations which could not be related to either soil temperature or moisture considered separately. There was, however, indication of an interaction between these factors. With high soil moisture the abundance of *F. culmorum* was positively correlated with rising temperature. At low moistures, on the other hand, there appeared to be strong inhibition above 8–9° C. These observations were substantially confirmed in an experiment in which unsterilized soil, taken from the rye-grass swards, was incubated under a range of moisture and temperature conditions. Again it was found that under low-moisture conditions there was marked reduction in the recovery of *F. culmorum* at temperatures above 10° C.

This effect of temperature is not a direct one, for the fungus grows well at high temperatures in soil with a low-moisture content. Its interaction with *Trichoderma viride*, which was abundant in the soil investigated, was, under low-moisture conditions, very striking and appears to account for the observed results. When *F. culmorum* and *T. viride* were grown together in soil at a moisture content of 40 per cent m.h.c., *F. culmorum* was dominant up to temperatures of 10° C. Above this temperature, however, it was completely suppressed by *T. viride*.

In a study of the pathogenicity of *F. culmorum* towards rye-grass a bacterium was found to be regularly present on the root surfaces, particularly of healthy roots. It was noted that this bacterium was antagonistic to *F. culmorum* on agar plates, and this aspect was investigated further. Surface sterilized seeds of rye-grass were sown in sterilized soil which had been inoculated with *F. culmorum* alone, *F. culmorum* + bacteria, and bacteria alone. It was found that under these conditions *F. culmorum* alone produced root browning accompanied by withering of the leaves, whereas in the presence of the bacterium the symptoms were substantially reduced. No pathological symptoms were observed in the seedlings raised in soil inoculated with the bacteria alone.

There can be little doubt that the bacterium is restricted to the rhizosphere. Although it was abundant on root surfaces it was only occasionally isolated from soil where its sporadic occurrence might well be expected since small root fragments would inevitably be present. The existence, in the rhizosphere, of organisms antagonistic to root pathogens has long been suspected and was apparently first suggested by Hiltner[1]; but there has been little experimental evidence to support this view. Hitherto the only cases of protective effects by rhizosphere populations have been attributed to the antagonistic effects of *T. viride*, which is preferentially favoured by the rhizosphere environment of certain plants[2].

The nature of the bacterium has been investigated and has been shown to be a polarly flagellated organism with the typical morphology and flagellation of a *Pseudomonas* and the biochemical reactions of a *Klebsiella aerogenes*[3].

E. Griffiths
M. A. Siddiqi

Department of Agricultural Botany,
University College of Wales,
Aberystwyth.
Aug. 5.

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Induced Swarming of a Nematode as a Means of Isolation

Since nematodes extracted from soil usually comprise a mixture of species, the isolation of a pure species population for experimental purposes is frequently a laborious procedure involving the picking of individual specimens from a suspension. The tendency of *Tylenchorhynchus martini* Fielding 1956 (Nematoda, Phasmidia) in aqueous suspensions to swarm (congregate in masses) has been used to obtain large numbers of this nematode relatively free of other species. In a series of tests, mixed populations of *T. martini* and other nematodes were extracted from soil around rice plants and incubated overnight in aqueous suspensions in Petri plates. The resultant masses of *T. martini* were observed under a dissecting microscope, carefully removed with a suction pipette and transferred to a small quantity of water in an Ehrlenmeyer flask. The nematodes were dispersed by shaking and then transferred to water in Petri plates for re-incubation. This procedure has been repeated several times with no apparent loss in swarming intensity, although

about 5 per cent of the population of *T. martini* in each test does not swarm and is lost in subsequent transfers. Suspensions of 50–100 thousand nematodes of 70–80 per cent *T. martini* have been purified in two serial swarmings to more than 99 per cent *T. martini*, with a virtual elimination of organic and mineral debris. Factors which stimulate swarming include a high proportion of active *T. martini* in the mixed population and a suspension density such that the individual nematodes of this species are in contact in a shallow aqueous layer. A range of room temperatures and of water reactions varying from *p*H 3·5 to 7·5 have not influenced swarming intensity.

JOHN P. HOLLIS

Department of Plant Pathology,
Louisiana State University,
Baton Rouge, Louisiana. Aug. 5.

Cardiac Glycosides and Thyroidal Iodide Transport

DURING the past few years, it has become apparent that an important action of the cardiac glycosides involves the transport of potassium ions into the cell. It has also become possible, by the use of potassium-depleted erythrocytes, to test the relative inhibitory potency of such glycosides or their aglycones *in vitro*[1,2]. Approximately 80 per cent of the potassium influx can be inhibited by the glycoside by an action that appears to involve competition by drug and potassium ions for the active transport site. The transport of chloride ions in the stomach appears to be similarly affected[3]. Since the maintenance of a concentration gradient for iodide by an 'active transport' is one of the fundamental properties of thyroid tissue, we determined whether or not iodide transport was likewise inhibited by the cardiac glycosides.

Sheep thyroid slices (~150 mgm.) were incubated for 70–90 minutes at 37° C. in Krebs–Ringer phosphate medium (3 ml.), *p*H 7·4, containing 10^{-3} *M* 1-methyl-2-mercaptoimidazole, to block organic iodine formation, and 0·2–0·3 μc. of carrier-free iodine-131. The iodine-127 content varied from 0·15 to 0·20 μgm. per 100 ml. Cardiac glycosides were dissolved in absolute ethanol, diluted with water and added to the flasks as 0·1 of the final volume to yield a final ethanol concentration of 1–2 per cent. Controls received equivalent quantities of ethanol. Results are expressed as the ratio of iodine-131 content per gram of tissue to the iodine-131 content per ml. of medium (*T*/*M* ratio).

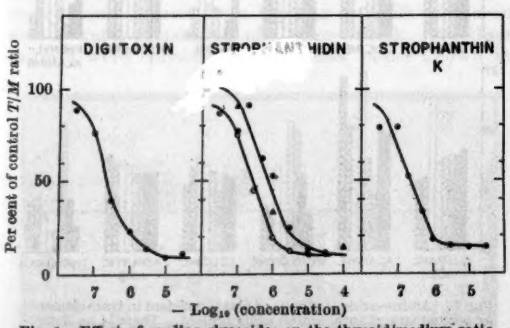

Fig. 1. Effect of cardiac glycosides on the thyroid/medium ratio (*T*/*M* ratio) of iodine-131 in sheep thyroid slices. The control *T*/*M* ratios varied from 12–25: ● and ▲, experiments 1 and 2, fitting approximately the same curve; ○, experiment 3

Table 1. INHIBITION OF IODIDE TRANSPORT BY CARDIAC GLYCOSIDES

	Concentration for 50 per cent inhibition of the *T*/*M* ratio* (*M*)	
	Sheep thyroid slices	Rat tumour slices
Digitoxin	2×10^{-7}	$1{\cdot}5 \times 10^{-4}$
Strophanthidin	$2{\cdot}5 \times 10^{-7}$	9×10^{-4}
Strophanthin *K*	2×10^{-7}	

* Incubated at 37° C. under air in Krebs–Ringer phosphate buffer, *p*H 7·4, for 70–90 min.

The results, depicted in Fig. 1, demonstrate that two glycosides, digitoxin and strophanthin *K*, as well as the aglycone strophanthidin, all depress the *T*/*M* at concentrations comparable to those found to inhibit potassium transport. For strophanthidin, the results of three separate runs show the variation in responsiveness that occurs between different batches of glands. The inhibitory potency of the drugs can be compared at the point of 50 per cent inhibition (Table 1). The order compares more closely with that of Solomon *et al.*[2] than that of Kahn and Acheson[1]. The concentrations for 50 per cent inhibition are probably somewhat lower, since the curves do not go below 10 per cent of the control *T*/*M* value (Fig. 1).

We have recently described a thyroid tumour in rats in which the iodide-concentrating mechanism survived without the further steps in hormone synthesis[4]. As shown in Table 1, the iodide concentrating mechanism of these tumours is about 1,000-fold less sensitive to the cardiac glycosides than is normal sheep thyroid tissue. Another tissue that normally maintains an iodide gradient is the submaxillary gland of mice[5]. Preliminary experiments with submaxillary glands of *C3H* mice have yielded variable results: no inhibition at 10^{-4} *M* strophanthidin and 30 per cent inhibition with 10^{-4} *M* digitoxin. We were, however, not able to obtain mice with salivary *T*/*M* ratios as high as those previously reported.

The above results indicate that thyroid tissue transports iodide by a system that is sensitive to inhibition by the cardiac glycosides. The kinetics of the inhibition and the relation of iodide to potassium transport are now under investigation.

We should like to thank Dr. J. E. Rall for his advice during the course of this work, and I. L. Cooperstein for permitting us to see the manuscript of ref. 3 prior to publication.

J. WOLFF
J. R. MAUREY

National Institute of Arthritis and
Metabolic Diseases,
National Institutes of Health,
Bethesda, Maryland.

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Spontaneous Electrical Activity in the Denervated Muscles of the Cockroach *Periplaneta americana*

SPECIAL features of the anatomy and physiology of the invertebrate neuromuscular junction raise the question of to what extent the changes caused by denervation of the insect muscles are analogous to those in the denervated muscles of vertebrates.

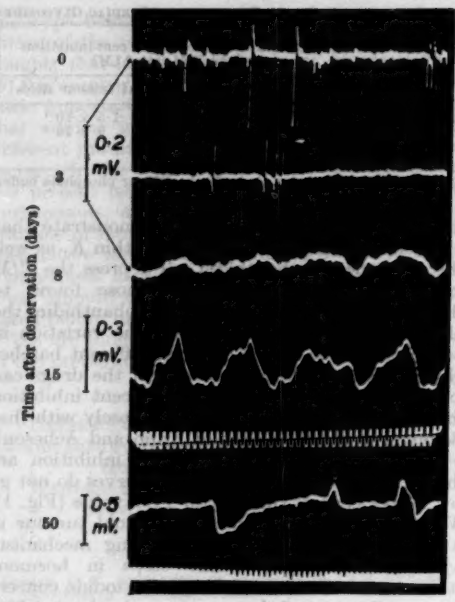

Fig. 1. Development of spontaneous electrical activity in the femoral muscles of the cockroach after denervation. Time scale: 500/sec.

Experiments were carried out to ascertain whether denervation of insect muscle is followed by spontaneous activity comparable with the well-known fibrillations in the denervated striated muscles of vertebrates. Voskresenskaya[1] described rhythmical spontaneous twitches in the extremity of the locust as early as the first day after denervation. Recently, Case[2], in visual observations of the denervated muscles of the abdominal spiracles of the cockroach, found "nearly continuous slow contractions and relaxations of small groups of muscle fibres" and called this phenomenon fasciculation. In the present experiments, the electrical activity of the denervated insect muscle was studied.

Adult males of *Periplaneta americana* (L.) were used. One extremity was denervated by cutting the nerves between the metathoracic ganglion and the left leg. Action potentials were recorded for the coxal and femoral muscles 0, 3, 8, 15 and 50 days after denervation. Monopolar insulated steel electrodes with a tip of approximately 35μ inserted through the chitin were used throughout the experiments. To prevent artefacts due to possible movements of the intact parts of the animal, the recording was performed on a metathoracic preparation obtained by isolating the metathorax with the extremities from the abdomen and the rest of the thorax. The open tracheæ ensured good metabolic conditions in this preparation.

Immediately after denervation, spontaneous electrical activity appeared as frequent pulses of short duration. Their duration (0·6–0·8 msec.) suggests their nervous origin. They were less frequent on the third day after denervation and from the eighth day they were replaced by a new type of activity, which was characterized by waves of 6–14 msec. duration with superimposed deflexions of 3–5 msec. The large waves became more numerous and increased in amplitude with time after denervation. The activity was irregular, of varying amplitude and disappeared and reappeared gradually at irregular intervals of 0·5–3 min. (50 days after denervation distinct long-duration spikes could be distinguished in the record, but at this time interference due to re-innervation cannot be excluded[3].)

It is presumed that the observed potential changes are an expression of spontaneous activity in the denervated muscle fibres with a varying degree of synchronization. They differ from the electrical activity of denervated vertebrate muscles, as was shown by a recording from the denervated tibial muscle of the rat using the same electrode, where only the usual short-duration spikes of individual fibres could be detected. When evaluating the denervation activity in the cockroach, it must be remembered that this can be the result of simultaneous potential changes in the various elements (slowly and fast contracting fibres) of the insect muscle.

R. Beránek

Institute of Physiology,
Czechoslovak Academy of Sciences,
Prague.

I. Novotný

Biological Faculty, Charles University,
Prague. July 28.

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Influence of Trace Elements upon Plant Protein Composition

Mulder's work[1] has shown that whereas macro-elements did not alter the amino-acid composition of potato protein, they did significantly alter the amino-acid composition of its soluble non-protein nitrogenous fraction. The influence of micro-nutrients on the amino-acid composition of plant proteins has not been properly studied, although there is evidence which suggests it might be appreciable[2]. There is, in fact, reason for believing that micro-elements may alter the relative proportions of the amino-acids in

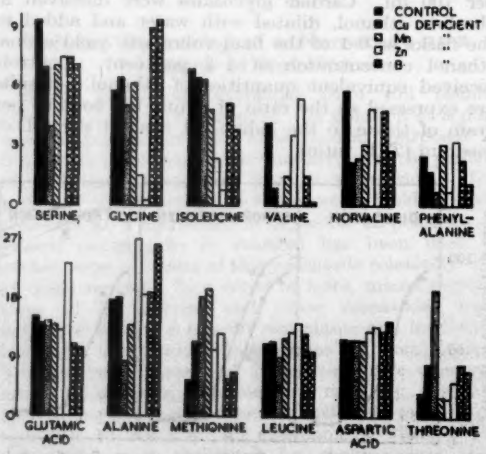

Fig. 1. Amino-acids of digests of plants deficient in trace elements as percentages of total amino-acids present. There is no detectable valine in the copper-deficient plant. One of the serine samples in the zinc-deficient plants was lost. Only single estimations of copper- and manganese-deficient plants were undertaken

plant proteins. If such is the case, this effect would be of great interest in animal and human nutrition, especially since deficiencies in trace elements are widespread[3], as is infant protein malnutrition (kwashiorkor), which appears to be primarily the result of amino-acid deficiency[4].

In a pilot study, beans (variety, Victory Dwarf Bush) were grown on Robbins's nutrient solution[5], and on this solution lacking in boron, copper, manganese and zinc respectively. They were harvested when the first fruits appeared, and the proteins were extracted according to the method of Lugg[6]. The proteins were hydrolysed for 12 hr. in 6 *N* hydrochloric acid and the amino-acid content of the hydrolysate was estimated by a semi-quantitative chromatographic method devised by one of us (H. E. S.). The results are recorded in Fig. 1.

There are indications in this preliminary investigation suggesting that beans grown in a copper-deficient medium have a higher concentration of threonine and perhaps a lower concentration of valine in their protein than normal, whereas beans grown in a zinc-deficient solution have proteins with a very low level of glycine and perhaps *iso*leucine. Confirmatory experiments are in progress on these and other plants.

We acknowledge the technical assistance of Misses H. Lenz and N. Posthumus.

K. H. Schütte

Department of Botany,

H. E. Schendel

Department of Medicine,
University of Cape Town.

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Retention of Food Residues in the Midgut by Nymphs of the Milkweed Bug, *Oncopeltus fasciatus* Dall.

During experiments on the nutrition of *Oncopeltus fasciatus*, in the course of which both nymphs and adults ingested solutions containing a fine suspension of Chinese black, it was noticed that only the adults voided black fæces, and that newly moulted adults voided a particularly thick black mass within two days of ecdysis. In a confirmatory experiment, the insects were reared from the egg on milkweed seed and a water-suspension of Chinese black, and their excreta were collected on the filter paper shown in Fig. 1. By the time the first adults had appeared, only watery material had been excreted, whereas three days later, when nearly all had moulted, black fæcal masses were evident.

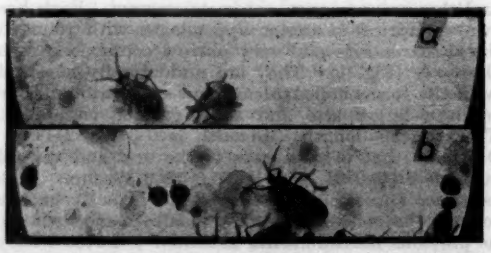

Fig. 1. The accumulated excreta of *Oncopeltus* reared on milkweed seed and a water-suspension of Chinese black: (*a*) just before and (*b*) after the majority had become adult

Examination of the nymphs showed that particles of Chinese black became concentrated in the third region of the midgut. It was also found that in mature nymphs which had fed on an abundance of milkweed seed, whether or not they had ingested Chinese black, this region was distended, had a flourishing bacterial flora, and contained a large droplet of oil. The short, narrow region (which has been called a 'fourth stomach'[1]) connecting the third region of the midgut to the 'pylorus' appeared, at this stage, to be histologically fully formed, but closed, although traces of a lumen could be made out along most of its length. In adults, the third region of the midgut did not reach the dimensions of that in the mature nymph, its bacterial flora was greatly depleted, and it was never found to contain a droplet of oil.

It appears, therefore, that in *Oncopeltus* the residues of nymphal meals accumulate in the third region of the midgut, to be voided soon after the final ecdysis. This does not seem to have been recognized previously, possibly because the nymphs excrete drops which contain reddish-brown solid matter, and these can be mistaken for true fæces, although they lack the thicker consistency of the latter and almost certainly originate entirely in the Malpighian tubes.

Many phytophagous Heteroptera possess special features associated with the last region of the midgut, such as cæca or inwardly directed filaments[2], which are lacking in *Oncopeltus*. It would be interesting to know, therefore, whether, in these cases, food residues are also retained in the nymphal midgut, since it is possible that the retention in *Oncopeltus* is concerned with ensuring the efficient extraction of nutrients from a simpler form of gut.

P. W. Miles*

Zoological Laboratory,
Cambridge. Aug. 5.

* Present address: Waite Agricultural Research Institute, University of Adelaide.

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Composition of the Cell Wall of *Staphylococcus aureus* 209P

Recently, Park and Strominger[1] assumed that uridine-diphosphate compounds extracted from cells of *Staphylococcus aureus H* treated with penicillin are the precursors of the cell wall, because the cell wall and one of their compounds contained muramic acid, alanine, glutamic acid and lysine in the ratio of 1 : 3 : 1 : 1. But the cell wall also contained glycine (ratio 3 : 8).

We found[2] that similar uridine-diphosphate compounds were accumulated in another strain of *S. aureus* treated with penicillin, but these compounds contained five amino-acids: alanine, glycine, lysine, aspartic and glutamic acid in various combinations. As it seems probable that there is a certain variation in the amino-acid composition of cell walls among different strains of a single bacterial species[3], a study was made of the amino-acid composition of the cell wall of the present strain to confirm the assumed correlation between the accumulated compounds and cell walls.

Table 1. Amino-acid Content of Hydrolysates from the Bacterial Cell Wall of *S. aureus* 209*P*, expressed in No. of Molecules (Muramic Acid referred to as 1)

	Molecules		Molecules
Muramic acid	1·0	Aspartic acid	0·6
Alanine	1·6	Leucine	0·8
Lysine	1·0	Serine	0·7*
Glutamic acid	0·8	Threonine	0·3
Glycine	2·3	Valine	0·4

* This is probably a maximum value, since serine was only incompletely separated from glycine on the paper chromatogram.

Using the method of Cummins and Harris[4], cells of *S. aureus* 209*P* were ruptured with a Mickle disintegrator, the homogenate was centrifuged off the intact cells at 3,000 r.p.m., and the supernatant was further centrifuged at 10,000 r.p.m. The precipitated cell-wall fraction was treated successively with trypsin and pepsin, and hydrolysed with 6 *N* hydrochloric acid for 6 hr. at 120° C. Amino-acids in the hydrolysate were separated by two-dimensional paper chromatography using the method of Levy and Chung[5]. Ninhydrin-positive spots were cut out from the paper, eluted, and determined by the method of Rosen[6].

As shown in Table 1, not only muramic acid, alanine, lysine and glutamic acid but also glycine, aspartic acid and leucine (and/or *iso*leucine) were predominant. The undigested cell-wall fraction contained considerable amounts of valine and threonine, and the whole cells contained also proline, phenylalanine, histidine, methionine and tyrosine. This evidence shows that the same five amino-acids are found both in the cell wall and in the present compounds as main components. Furthermore, it was found that the main species of peptides linked with uridine-diphosphate-N-acetyl muramic acid were peptides composed of three alanine, two lysine and one glutamic acid residues, and of two alanine, two lysine, two glycine, one glutamic acid and one aspartic acid residues (Ito, E., Ishimoto, N., and Saito, M., unpublished work).

This evidence seems to support the assumed correlation between the accumulated compounds and cell walls.

Nobutoshi Ishimoto
Masahiro Saito
Eiji Ito

Department of Chemistry,
Faculty of Science,
Hokkaido University,
Sapporo, Japan.
July 7.

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Sagitta as a Host of Larval Trematodes, including a New and Unique Type of Cercaria

It has long been known that *Sagitta* may occasionally foster young trematodes, but we have learned very little since Müller, Busch, Leuckart and Pagenstecher made discoveries of this kind more than one hundred years ago. During the present century, Miss M. V. Lebour found two species of Digenea in *Sagitta bipunctata*[1]. Small *Derogenes varicus* (Hemiuridae) were seen near the ovary, generally singly, and post-larval *Opechona bacillaris* (Allocreadiidae) were seen in the gut as well as near the ovary, indicating that migration into the cœlom takes place through the wall of the intestine. Young forms similar to *Derogenes* have been found in the body cavity of unspecified chætognaths in South Australia, and small yellow cercariæ in the cœlom of *Sagitta enflata* were recorded but not described. Small appendiculate Hemiurids have been seen in *S. elegans* at Woods Hole and in *S. enflata* from the Bay of Algiers, and forms identified as small *Hemiuris communis* have appeared in *S. elegans* and *S. setosa* from Northumbrian plankton. Young forms of a rarer trematode—some unnamed member of the Accacoeliidae and possibly a species of *Tetrochetus*—have been found in *S. enflata* from the Indian Ocean near Madras[2].

Any trematode larvæ which gain entry into *Sagitta* may, it seems, with equal facility enter planktonic cœlenterates and copepods, and post-larval Hemiurids, Accacœliids and other Digenea have been found in such hosts. Heavy infections have been reported in siphonophores. In an infection of *Physalia utricula* with an unnamed Accacœliid near Japan, "les plus petits spécimens . . . de 5 mm. de long, ont déjà abrité un grand nombre de parasites dans leur polypes en développement, ainsi que dans diverses parties de leur poche aérienne"[3]. It is difficult to see how a fish could be infected through the agency of such a virulent vector, but it is evident that close co-operation between students of the plankton and parasitologists is necessary for the acquisition and study of the life histories of some of these elusive trematodes.

By the kindness of Dr. John Colman, I have been privileged to examine a number of parasitized *Sagitta* collected on the *Rosaura* Expedition[4]. Two specimens of *S. tenuis*—one from Barima River, Morowhana, and the other from Demerara River, Georgetown—contained a small appendiculate Hemiurid, which is probably a species of *Hemiuris*, and a very young larval cestode respectively. One *S. hexaptera* from the northern edge of the North Equatorial Current contained numerous multinucleate, non-helminthic parasites which are as yet unidentified. Twelve other *S. hexaptera*, collected in various localities in the Caribbean—between Cuba and Jamaica, off Roatan Island, some 250 miles N.N.W. of Colon, and off Puerto Columbia—contained a total of 15 larval trematodes of the same kind. Three hosts had two parasites each, the remaining nine hosts one apiece. Two of the larvæ were located in the intestine, the remaining 13 in the body cavity.

These larvæ have been seen only once hitherto and they are cercariæ, not metacercariæ. Each has two cylindrical posterior appendages with rounded distal extremities and very narrow connexions with the body (Fig. 1). They are undoubtedly identical with the forms called *Metacercaria owreae*, but they reach a larger size and show greater variability. The range of length × breadth is 0·3 × 0·2–1·2 × 0·7 mm., that of diameters of the oral and ventral suckers 0·07–0·24 mm. and 0·12–0·37 mm. The posterior appendages are not almost exactly half as long as the body in large and small specimens alike, as previous measurements indicated[5]; instead, they are of extremely variable length (0·25–1·45 body-length) and not proportionate to the size of the body.

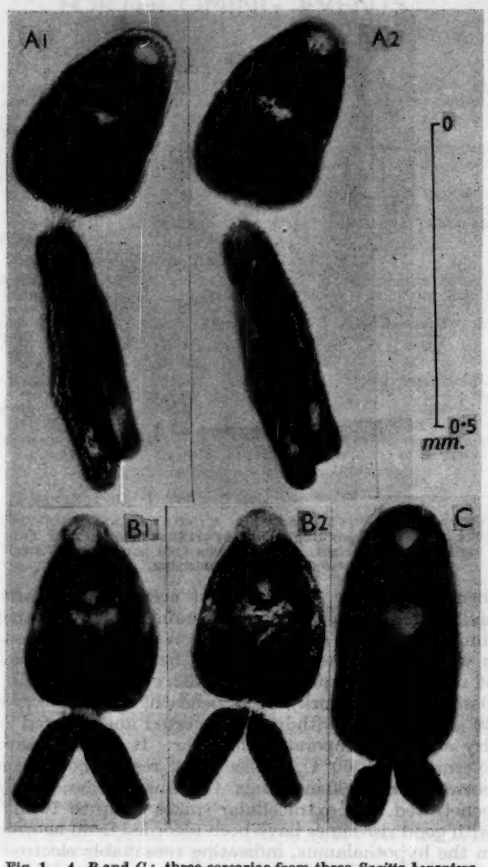

Fig. 1. *A*, *B* and *C*: three cercariae from three *Sagitta hexaptera* taken 250 miles north-north-west of Colon. In *A*1 and *B*1 the focus is on the ventral sucker; in *A*2 and *B*2 on the pharynx and the gut caeca. Actual body-lengths: *A*, 0·34 mm.; *B*, 0·33 mm.; *C*, 0·43 mm. Note the varying length of the posterior appendages; 0·4 mm. in *A*, 0·2 mm. in *B* and 0·1 mm. in *C* (approx.)

I can confirm the observation that a prolongation of each cæcum extends into the corresponding appendage—so that the term 'tail' is inadmissible—and I affirm that the corresponding adult must therefore possess a pair of anal openings. This unusual condition is known in only a few trematodes, but among these are the seven genera of the Accacoeliidae[6], and in this instance it must arise as a consequence of loss of the appendages during post-larval development. Another notable character, not observed hitherto, is the existence of short anterior extentions of each cæcum in the form of six very short and rudimentary diverticula, which form a kind of rosette at the tip. These are indications that this larval Accacoeliid probably belongs to the genus *Accacladocoelium* Odhner, 1928, but to which of four known species is uncertain because of the state of immaturity, although *A. petasiporum* Odhner, 1928, may be ruled out by the form of the ventral sucker. Older specimens with shrunken appendages or without appendages show peculiarly fine annulations indicative of *A. nigroflavum* (Rud., 1819).

New specimens of *Cercaria owreae* will not be easy to find, unless the following point regarding distribution proves helpful. Hitherto, only 8 specimens were found in 5,767 specimens of *Sagitta enflata*, *S. hexaptera* and *S. lyra*. The total incidence of infection was given as 0·14 per cent, the incidence for respective species 0·09, 0·14 and 1·15 per cent, no other figures being given. My specimens were obtained from 290 *S. hexaptera*, but 109 hosts of this aggregate which were collected off the north coast of Brazil and in the Central Atlantic did not contain any parasites; all 15 larvæ were obtained from 181 *S. hexaptera* collected in the Caribbean localities specified. Moreover, no parasites were found in 141 *S. enflata*, 110 *S. lyra* and 167 *S. maxima*, which are all transparent species in which any parasites could have been readily seen. The *Rosaura* hauls of *S. hexaptera* were made in open nets between 1,250 m. and the surface, although this is a species living mainly between the surface and 100 m. The first intermediate host of this trematode is presumably a bottom-dwelling mollusc of coastal waters, and the posterior appendages are probably organs of flotation which bring emergent larvæ into the realm of the plankton. The re-appearance of the pelagic larvæ may be anticipated in the Caribbean plankton and that of the Florida Current, probably also in that of the Gulf of Mexico and other localities. They must be sought as free forms, as parasites of species of *Sagitta* and as parasites of other planktonic animals such as coelenterates and copepods. The definitive host is most probably the sunfish *Mola mola* (L.) but it may well be some other oceanic fish which feeds on plankton. Infection will result from the ingestion of planktonic vectors containing larvæ, possibly from the ingestion of free forms.

I wish to thank Mr. A. T. Green of King's College for taking the photographs.

Ben Dawes

Department of Zoology,
King's College,
London, W.C.2.
July 19.

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The Saw-Fish of Sierra Leone

There are three species of saw-fish which are occasionally caught along the coast of Sierra Leone. These are *Pristis pectinatus* (Latham), *Pristis pristis* (Linn.) and *Pristis peroteti* (M. and H.). The majority of captures have been made within the rivers, river mouths and from the open beaches; but as few of the captures have been subjected to any critical examination, practically nothing is known about the habits of saw-fish in Sierra Leone waters.

According to Copley[1], *Pristis pectinatus* and *Pristis peroteti* regularly ascend quite a long way up the Sherbro River when the river is in flood, and return to the sea on the falling of the waters. Whether this is a spawning migration has not yet been confirmed. The same species have also been caught in the Sierra Leone River, which is about 60 miles to the north of the Sherbro River, by the West African Fisheries Research Institute during trawling operations during 1952–57. They are also to be found more frequently in the Sierra Leone River when the river is in flood.

Saw-fish sometimes become entangled in the nets of the local fishermen, causing considerable damage to both nets and catch. For this reason, they are

regarded as pests rather than a source of food, in spite of the fact that there is a ready market for the flesh. In the Keta district, Ghana, the 'saw' is removed and used for fetish purposes, being specially coloured alternately red, white and blue colours[1]. It does not, however, appear to have any religious significance for the fishermen of Sierra Leone.

During the course of a fish-marking cruise in May, a male specimen of *Pristis peroteti* was caught in the Sierra Leone River, about ten miles from the mouth. This fish weighed 257·6 kgm. and measured 4·42 m. from the tip of the 'saw' to the end of the caudal fin. The liver yielded more than 13 litres of oil, and within the stomach was found a 52-cm. 'whiting' (*Cynoscion brachygnathus*, Bleek), which was in a fair state of preservation and showed wounds caused by the 'saw'. There was a deep cut on the left side, about 19·5 cm. long, in front of the operculum and a stab wound in the flesh of the back in front of the dorsal fin. There was no other food material either in the stomach or the intestine of this saw-fish.

Although saw-fish may occasionally use the 'saw' as a defensive weapon, its main purpose is to obtain food. This it does by grubbing about on the bottom in its search for small fish and crustaceans lying buried there. It may also use the 'saw' as a weapon of attack, as the present instance shows; but it probably does so only rarely in the estuaries of Sierra Leone as the water is usually very turbid. In this connexion, it is interesting to note that the specimen under consideration was caught at the end of the dry season (May), when the waters of the Sierra Leone River are at their clearest.

J. C. D. Watts

Fisheries Development and Research Unit,
Freetown, Sierra Leone.

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A Simple Microelectrode for recording from the Central Nervous System

Metal microelectrodes suitable for recording from single cells externally or after penetration have been described by various authors[1–3]. The procedures of Svaetichin[2] and Grundfest[1] call for special apparatus, and the method of Hubel[3], employing tungsten, in our hands gives rather noisy electrodes which are difficult to straighten. Steel electrodes, which have the advantages of low noise, low electrode potential, ease of straightening and, in particular, offer the possibility of accurate localization of the recording site, may be made as follows.

Stainless steel insect pins size 00, which are about 1·5 in. long (Clay-Adams Co.), are used. Using a filament transformer of 7 V. output, regulated by a variable auto-transformer, the terminal 1 cm. is thinned with alternating current against a carbon electrode dipped in concentrated hydrochloric acid. The surface of the acid is covered with xylol to limit the highly corrosive spray. The voltage is then reduced to about half and the tip quickly agitated up and down through the xylol–acid interphase. A highly polished and very sharp tip is produced. A terminal thickness of less than 0·5μ is readily obtained (Fig. 1*b*). Residual acid must be removed quickly to avoid pitting, so the electrode is quickly dipped in strong sodium carbonate solution and

Fig. 1*a*. Site of recording marked by prussian blue reaction. Two earlier tracks to right. Mark = 200μ (100 μamp. for 10 sec.)
Fig. 1*b*. Electrode tip. Oil immersion. Mark = 2μ

then successively in 1 per cent acetic acid, alcohol and xylene. Insulation is obtained in a manner similar to Hubel's technique[3]. While the electrode is wet with xylene, it is pushed beneath the surface of unthinned lacquer (Insl-*X* *E*33 clear, Insl-*X* Co., Ossining, New York), held beneath the surface for 30 sec., quickly withdrawn, inverted and allowed to dry at room temperature for 2 hr. It is then baked overnight at 60° C. Electrode resistance ranges between 5 and 100 megohms. Cells may be successfully penetrated, and extracellular spikes of up to 10 mV. with good rise times have been recorded from neurons in the hypothalamus, indicating reasonable electrode capacitance. The recording site may be marked by a current of about 2 μamp. for 15–30 sec. If the electrode is made the anode, a small amount of iron is lost. (The insulation may then be observed under the microscope as a transparent terminal core.) The animal is fixed in 10 per cent formalin with about 1 per cent potassium ferrocyanide added. A prussian blue spot is obtained. The large deposit shown in Fig. 1*a* was made by a much larger current (100 μamp. for 10 sec.), but this is unnecessarily large.

It may be noted that if the lacquer advised is not available, a lacquer of high viscosity with a tendency to form a surface pellicle rapidly is desirable. This avoids beading and, while the electrode is inverted, the coat becomes thin as the central lacquer is still capable of flowing away from the tip beneath the surface pellicle. Lacquer shrinkage in the final setting determines the size of the exposed tip.

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J. D. Green

Department of Anatomy,
School of Medicine,
University of California,
Los Angeles 24.
July 28.

[1] Grundfest, H., Sengstaken, R. W., Oettinger, W. H., and Gurry, R. W., *Rev. Sci. Inst.*, **21**, 300 (1950).
[2] Gray, J. A. B., and Svaetichin, G., *Acta Physiol. Scand.*, **24**, 278 (1951).
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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, October 6

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE, PHILOSOPHY OF SCIENCE GROUP (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. O. Kapp: "Space" (Chairman's Address).

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. Keith H. Best: "Bridge Over the River Kwai".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. W. S. Wood: "Some Developments in the Field of Chemical Propellents" (Chairman's Address).

Tuesday, October 7

SOCIETY FOR VISITING SCIENTISTS (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "The Metric System in Great Britain". Chairman: Sir Charles Darwin, F.R.S. Speakers: Dr. A. H. Hughes, Sir Graham Savage, C.B., and Dr. H. Barrell.

Wednesday, October 8

PHYSICAL SOCIETY, COLOUR GROUP (in the Physics Department, Imperial College, Imperial Institute Road, London, S.W.7), at 3 p.m.—Prof. H. Munro Fox, F.R.S.: "The Nature of Animal Colours"; Dr. M. H. Pirenne: "Colour and Perspective in Painting".

BRITISH PSYCHOLOGICAL SOCIETY, SOCIAL PSYCHOLOGY SECTION (at Birkbeck College, Malet Street, London, W.C.1), at 8 p.m.—Dr. F. E. Emery: "Inducing Technological Change".

Thursday, October 9

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. L. S. Penrose, F.R.S.: "Mutation in Man".*

PHYSICAL SOCIETY, OPTICAL GROUP (at Imperial College, London, S.W.7), at 2.30 p.m.—Mr. H. H. Hopkins: "Frequency Response Methods for Assessing Optical Image Quality"; Mr. A. M. Goodbody: "Study of the Influence of Spherical Aberration and Coma on Frequency Response"; Mr. D. Kelsall: "Measurement of Frequency Response Curves in the Presence of Spherical Aberration using an Automatic Recording Instrument".

PHYSICAL SOCIETY, LOW TEMPERATURE GROUP (at the Royal School of Mines, Prince Consort Road, London, S.W.7), at 4 p.m.—Dr. R. T. Webber: "Cryomagnetic Research".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. S. E. Goodall: Inaugural Address as President.

ROYAL AERONAUTICAL SOCIETY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 6 p.m.—Mr. J. C. Floyd: "Some Aspects of Canadian All-Weather Fighter Development" (Fourteenth British Commonwealth Lecture).

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, Portland Place, London, W.1), at 6 p.m.—Dr. A. E. Cutler: "Aircraft Flight Simulators".

Friday, October 10

BRITISH MYCOLOGICAL SOCIETY (in the Large Physics Theatre, Birkbeck College, Malet Street, London, W.C.1), at 11 a.m.—Scientific Papers.

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 11 a.m.—Symposium on "Variations in Anatomical Structure and the Causes to Which They are Due".

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Four papers on Astronomical Research.

PHYSICAL SOCIETY, ACOUSTICS GROUP (in the Physics Department, Imperial College, South Kensington, London, S.W.7), at 5.30 p.m.—Dr. A. J. King: "Vibration—Its Causes, Measurement and Reduction".

Saturday, October 11

BRITISH PSYCHOLOGICAL SOCIETY (in the Gustave Theatre, University College, Gower Street, London, W.C.1), at 2.35 p.m.—Prof. Wolfgang Köhler (Swarthmore College, U.S.A.): "The Present Situation in Psychophysics".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (with a science degree with special qualifications and postgraduate experience in entomology or helminthology) IN ZOOLOGY—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (October 10).

LECTURER (Grade III or II) IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, The University, Bristol 8 (October 13).

LECTURERS (2) (with experience of applied electronics or of electrical plant and control systems) IN ELECTRICAL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (October 13).

DEMONSTRATOR (primarily zoological) IN THE DEPARTMENT OF BIOLOGY—The Registrar, University College of North Staffordshire, Keele, Staffs (October 14).

READERS (2) IN PHYSICS—The Registrar, Panjab University, Chandigarh-3, India (October 15).

VOKES RESEARCH FELLOW IN THE DEPARTMENT OF MECHANICAL ENGINEERING, to undertake research in the field of filtration, the separation of mixtures, or allied problems in fluid mechanics—The Secretary and Registrar, The University, Southampton (October 18).

SENIOR RESEARCH OFFICER or RESEARCH OFFICER (qualified zoologist with experience of fisheries research) IN THE FISHERIES RESEARCH UNIT within the Department of Zoology, University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, October 30).

LECTURER IN GEOGRAPHY AND GEOLOGY at the University of Natal, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, October 31).

LECTURER IN PSYCHOLOGY at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, October 31).

LECTURER IN THE DEPARTMENT OF MECHANICAL ENGINEERING, University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, October 31).

SENIOR RESEARCH FELLOWS (2), and a RESEARCH ELECTRONICS ENGINEER IN THE NUCLEAR PHYSICS RESEARCH UNIT, Department of Physics, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, October 31).

REGIUS CHAIR OF MATERIA MEDICA in the University of Aberdeen—The Private Secretary, Secretary of State for Scotland, St. Andrew's House, Edinburgh 1 (November 10).

ASSISTANT DIRECTOR OF RESEARCH IN GENERAL SURFACE CHEMISTRY AND COLLOID SCIENCE—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield Road, Cambridge.

ASSISTANT PHYSICIST (with a first- or second-class honours degree in physics) at the Regional Laboratories, Royal Victoria Infirmary, Newcastle-upon-Tyne, for duties relating primarily to the uses of high energy radiation for medical purposes—The Secretary, Newcastle Regional Hospital Board, Newcastle-upon-Tyne 6.

BIOCHEMIST (with a degree in biochemistry, chemistry or physiology) IN THE PATHOLOGICAL LABORATORY, Dulwich Hospital—The Group Secretary, Camberwell H.M.C., Dulwich Hospital, London, S.E.22.

BIOCHEMIST (with a knowledge of bacteriology) IN THE BACTERIOLOGY DEPARTMENT, for research on the genus *Lactobacillus* and similar organisms—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 58/26.

ENGINEER or CHEMICAL ENGINEER, to improve design, and test equipment for extracting and processing leaf protein—The Secretary, Rothamsted Experimental Station, Harpenden, Herts.

LABORATORY TECHNICIAN (male, experienced in rock sectioning, etc., photography and general laboratory maintenance) IN THE NEW DEPARTMENT OF GEOLOGY—The Secretary, The University, Exeter.

LECTURER (preferably with the ability to teach educational psychology) IN EDUCATION in the Training Department—The Warden, Goldsmiths' College (University of London), New Cross, London, S.E.14.

LECTURER (with a degree or professional qualifications) IN SPECTROSCOPY; and LECTURERS (2) IN ELECTRONICS—The Principal, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3.

ORGANIC CHEMISTS (2), and a BIOCHEMIST (with at least a first- or second-class honours degree and special aptitude for exacting preparative chemistry), to work on the labelling of complex compounds with radioactive isotopes—The Personnel Officer (1225Y/34), The Radiochemical Centre, Amersham, Bucks.

PHYSICIST (with considerable experience in radiological protection work), to assist the Superintendent Health Physicist—The Senior Recruitment Officer, Atomic Weapons Research Establishment, Aldermaston, Berks, quoting Ref. 2012/34.

PHYSIOLOGIST (or PHARMACOLOGIST) (with a Ph.D. or equivalent experience of research, and preferably experience in pharmacological assay, experimental physiology or electro-physiology) with the Medical Research Council in the Department of the Regius Professor of Medicine, for research into various problems connected with neurohumoral physiology—The Regius Professor of Medicine, Radcliffe Infirmary, Oxford.

POSTDOCTORAL RESEARCH FELLOW (BIOCHEMIST or CHEMIST), to conduct investigations into the chemistry and biochemistry of carbohydrate sulphate esters—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff.

REGIONAL TECHNICAL INFORMATION OFFICER (qualified in pure and applied science with a broad background of knowledge and experience)—The Principal, Welsh College of Advanced Technology, Cathays Park, Cardiff.

RESEARCH ASSISTANT (with a degree in physics or a degree in geology and a good knowledge of physics) IN PALÆOMAGNETISM, for work on the magnetic properties of rocks—The Registrar, The University, Birmingham 15.

RESEARCH ASSISTANT (with a good first degree in botany or horticulture, and preferably research experience and a higher degree), for work on the physiology of flowering in carnations—Prof. O. V. S. Heath, Department of Horticulture, The University, Reading.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

A Guide for Industry to the Clean Air Act 1956. Pp. iv+48. (London: Federation of British Industries, 1958.) 4*s*. [307

Library Association. Special Subject List No. 26: Radio Astronomy. Compiled by F. R. Taylor. Pp. 15. (London: Library Association, 1958.) 2*s*. 6*d*. [307

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Organic Chemical Reagents. Monograph No. 35 (1958): 2: 4–Xylenol: Reagent for Nitrate. By J. T. Yardley and T. H. Cooper. Pp. 4. (Chadwell Heath: Hopkin and Williams, Ltd., 1958.) [317

Commission for Technical Co-operation in Africa South of the Sahara: Scientific Council for Africa South of the Sahara. Publication No. 27: Symposium on Eichhornia Crassipes, Léopoldville, 1957. Pp. 38. Inter-African Phyto-Sanitary Commission. List of Specialists in the Phyto-Sanitary Field. Pp. 54. Inter-African Conference on Statistics, 2nd Meeting, Lourenco Marques. Pp. 39. (London: C.C.T.A. C.S.A., 43 Parliament Street, S.W.1, 1957, and 1958.) [317

Micro-Determination of Iodine in Biological Materials, with Select Bibliography. Pp. v+40. (London: Chilean Iodine Educational Bureau, 1958.) [18

Department of Scientific and Industrial Research. Road Research Road Note No. 1: Recommendations for Tar Surface Dressings. Third edition. Pp. 11. (London: H.M. Stationery Office, 1958.) 1s. 3d. net. [18

Medical Research Council of Ireland. Annual Report for the year ended December 31, 1957. Pp. 1+51. (Dublin: Medical Research Council of Ireland, 1958.) [18

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 983, Vol. 250 (31 July, 1958): An Experimental Study of Thermal Convection in a Rotating Liquid. By R. Hide. Pp. 441–478. 25s. No. 984, Vol. 250 (31 July, 1958): Uniform Asymptotic Expansions for Large Values of a Parameter. By F. W. J. Olver. Pp. 479–517. 12s. 6d. No. 985, Vol. 250 (31 July, 1958): Dynamic Properties of Resilient Materials: Constitutive Equations. By J. E. Adkins. Pp. 519–541. 7s. 6d. (London: Royal Society, 1958.) [18

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Beef Cattle Production: a Series of Lectures delivered at Massey Agricultural College. By Dr. M. A. MacDonald. Pp. iv+59. (Palmerston North: Massey Agricultural College (University of New Zealand), 1958.) [307

Canada. Department of Mines and Technical Surveys: Geological Survey of Canada. Bulletin 41: Fauna, Age and Correlation of the Jurassic Rocks of Prince Patrick Island. By Hans Frebold. Pp. vii+59 (18 plates). 1.50 dollars. Bulletin 42: Bryozoa (Mainly Trepostomata) from the Ottawa Formation (Middle Ordovician) of the Ottawa–St. Lawrence Lowland. By Madeleine A. Fritz. Pp. v+75 (30 plates). 1.50 dollars. (Ottawa: Queen's Printer, 1957.) [307

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Museum of Comparative Zoology at Harvard College. Breviora, No. 86 (May 29, 1958): A New Sicistine Rodent from the Miocene of Wyoming. By Craig C. Black. Pp. 7. No. 87 (June 20, 1958): An Embolomere Jaw from the Mid-Carboniferous of Nova Scotia. By Alfred Sherwood Romer. Pp. 8. No. 88 (June 30, 1958): A New Species of the Genus *Urotheca* (Serpentes: Colubridae) from Venezuela. By J. A. Roze. Pp. 5. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1958.) [58

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